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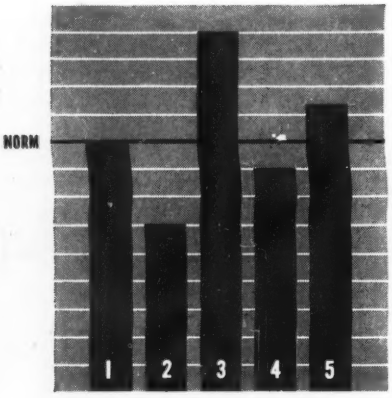

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ADRENAL CORTICAL TUMOR ASSOCIATED WITH CUSHING'S SYNDROME

REPORT OF A CASE WITH METABOLIC STUDIES AND REMARKS ON THE PATHOGENESIS OF CUSHING'S SYNDROME

EDWIN J. KEPLER, M.D.,* RANDALL G. SPRAGUE, M.D., O. THERON CLAGETT, M.D., MARSCHELLE H. POWER, PH.D., HAROLD L. MASON, PH.D., AND H. MILTON ROGERS, M.D.**

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THIS case report and the metabolic studies that accompany it are presented primarily because of their bearing on the pathologic physiology of Cushing's syndrome. The term "Cushing's syndrome" as used herein refers only to the clinical picture emphasized by Cushing. The term "Cushing's disease" will be used in a broader sense than we used it in previous publications so as to denote all cases of Cushing's syndrome in which an adrenal cortical tumor is not present.

REPORT OF CASE

A married woman aged twenty-six years first registered at the Mayo Clinic on September 3, 1943. There was nothing remarkable in her past medical history. On June 23, 1941, she consulted her physician because she was eight weeks pregnant. At that time she weighed 110 pounds (50 Kg.). The blood pressure expressed in millimeters of mercury, was 100 systolic and 60 diastolic, and the patient seemed to be a normal pregnant

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* Deceased since this paper was written.

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woman. Pregnancy proceeded uneventfully until December 22, 1941, when she was delivered of a premature infant who died shortly after birth. On February 21, 1942, she had one scant menstrual period. No further menstrual bleeding occurred until May, 1942, when she began to have daily spotting of blood. The following month a dilatation and curettage was performed. The endometrium was found to be "hyperplastic." After this operation, vaginal bleeding and menses ceased. In January, 1943, she had a renal

FIG. 1. Patient at time of initial examination. Note thin arms and legs, striae, protuberant abdomen and cervicodorsal hump.

colic on the right side and may have passed a stone. Investigation of the urinary tract revealed only hydronephrosis on the right.

About May, 1943, a new train of symptoms began. Hair began to appear on her upper lip, cheeks, legs and arms. The hair of the scalp began to fall out. Her face began to swell and her eyes seemed to protrude. A "bump" appeared on the back of the neck. Her friends noted that she "looked fatter" and that her "expression" was different. She became very nervous and apprehensive. One of the most distressing symptoms was muscular weakness, particularly of the legs. Walking and climbing stairs became difficult and she had to spend much of her time in bed. She also suffered from transitory attacks of

numbness of the entire right half of the body. In May, 1943, it was discovered that erythrocytosis (7,000,000 erythrocytes per cubic millimeter) was present. Because of this she was given medicine to "thin the blood," and in addition she was "treated with hormones." By August, 1943, she presented most of the symptoms of Cushing's syndrome, and she was referred to the Clinic with the suggestion that she might have a tumor of the adrenal cortex.

At examination, September 3, 1943, the patient's height was 66¾ inches (169.5 cm.) and her weight was 125 pounds (56.7 Kg.). The patient was pleasant and co-operative but obviously alarmed about her condition. She had thin arms and legs, a protruding abdomen and a moderate kyphosis (Fig. 1). The face was full, round and highly colored. Hair was present on the upper lip, the cheeks, arms and legs. At the temples, the hair line was receding and she seemed to be losing some hair at the crown of the head. Although the eyes were slightly prominent, the upper lids drooped. The corners of the mouth likewise drooped. Purplish striations were present on the buttocks. There was no acne. Large and deeply pigmented areolae made the breasts conspicuous. The labia majora were slightly edematous. The clitoris was normal in size.

On pelvic examination it was suspected that a retro-uterine mass might be present. Contrary to the uterine atrophy which is often found in cases of Cushing's syndrome, in this case the uterus seemed to be normal in size. Although the circumference of the arms and legs was reduced, the panniculus of fat was well preserved. The muscles felt soft and flabby. Muscular weakness was easily demonstrated in all four extremities. The extensor groups were weaker than the flexors, and the lower extremities seemed to be weaker than the upper. Tendon reflexes were all present. The blood pressure was 204 systolic and 110 diastolic. In the ocular fundi the arterioles were slightly narrowed and, in some, localized spastic constrictions were noted.

An excretory urogram showed slight caliectasis on the right side. Above the left kidney, which seemed to be displaced downward, there was suggestive evidence of a soft-tissue mass. These urologic findings suggested, but did not prove, the presence of an adrenal tumor on the left. Roentgenograms of the head, thorax and extremities did not reveal any abnormality. Evidence of a slight degree of osteoporosis was noted in the roentgenograms of the pelvis. The results of additional laboratory studies conducted at this and subsequent visits are recorded in Table 1.

After the initial clinical examinations were concluded, a diagnosis was made of Cushing's syndrome associated with tumor of the adrenal cortex.

In order to permit study of the metabolism of electrolytes and the urinary excretion of steroids, the patient was admitted to the metabolic unit of the Clinic. The observations that were made there are presented elsewhere in this report.

On October 15, 1943, after completion of the metabolic studies, both adrenal glands were explored surgically by way of bilateral posterolumbar incisions. Because the patient complained of pain in the right flank, the right adrenal gland was explored first. It appeared to be normal and was not disturbed. On the left side a large, fairly well-encapsulated adrenal tumor was encountered and enucleated without difficulty. After removal, the tumor measured 10 by 15 by 8 cm., weighed 410 Gm., was yellowish in color and was surrounded by a plexus of veins. The capsule was ruptured at several points.

In order to prevent the occurrence of acute adrenal insufficiency postoperatively, the patient was treated before and immediately after the operation with desoxycorticosterone acetate and adrenal cortical extract. Details of the treatment employed are recorded in Table 2.

Convalescence proceeded uneventfully, and three weeks after the operation the metabolic studies which had been instituted before the operation were continued. The patient remained under observation until December 3, 1943. After the operation there was a gradual decrease in the blood pressure, and prior to her dismissal the daily readings were normal, ranging from 120 to 130 systolic and from 60 to 70 diastolic. She was feeling very well. Her strength was returning. The arms and legs were beginning to take on their normal shape, and it was roughly estimated that the muscle tissue palpable in the calves had increased 30 to 40 per cent. Venipuncture no longer induced ecchymosis. Her facial contours were less round. New hair was appearing in the scalp and the excess hair on the thighs was disappearing. The skin lost its sandpaper appearance and texture and it appeared to be entirely normal, except for the purplish striae, which remained unchanged.

TABLE 1. LABORATORY FINDINGS

	September 1943 (before operation)	October-November 1943 (after operation)	January, 1945 (after recurrence of tumor)
Urine			
Specific gravity	1.026	1.017	1.017
Reaction	Alkaline	Acid	Alkaline
Albumin, grade*	2	1	2
Sugar, grade*	1	0	1
Microscopic:			
Pyuria, grade*	1	3	1
Other findings	Gram-positive cocci		Occasional erythrocyte
Blood			
Hemoglobin, gm.†	17.8	12.0	11.05
Erythrocytes‡	4,950,000	3,620,000	3,020,000
Leukocytes‡	10,200	8,000	11,700
Comment	§		
Urea, mg.†	23	23	38–106
Sugar, mg.†	83		
Hematocrit‖	56.9	35.7	46
Sodium, mEq.¶	146.0	140.0	149.0
Potassium, mEq.¶	4.4	4.7	2.2
Carbon dioxide, mEq.¶	34.2	28.4	39.2
Chloride, mEq.¶	96.0	104.0	92.0
Calcium, mg.**	9.84	9.7	
Phosphorus, mg.**	1.76	4.81	
Phosphatase, alkaline, Bodansky units**	4.0		
Protein, Gm.**	7.0		

TABLE 1. (*continued*)

	September 1943 (before operation)		October-November 1943 (after operation	January 1945 (after recurrence of tumor)
Miscellaneous				
	Sugar, mg.†			
Glucose tolerance	Blood	Urine		
Fasting	77	0		
½ hour	112	0		
2 hours	154	0		
3 hours	104	0		
Blood volume				
Plasma, c.c.	1,971 (37 cc. per Kg.)			
Whole blood, c.c.	4,584 (86 cc. per Kg.)			
Gonadotropic hormone (prolan), rat units per liter (Frank technic)	Less than 5			
Urinary 17-ketosteroids, mg. per 24 hours	136		2.7	110 (60% beta fraction)†† 314‡‡
Urinary estrogens, rat units per 24 hours	9,400		43	5,000+
Sedimentation rate, mm. in 1 hour	10			
Basal metabolic rate, per cent	0		+15	

* Graded on the basis of 1 to 4, in which 1 represents the mildest and 4 the most severe condition.

† Per 100 c.c. of blood.

‡ Per cubic millimeter of blood.

§ Increased regeneration, piling of erythrocytes and leukocytes; suggest polycythemia vera.

‖ Per cent of cells.

¶ Per liter of plasma.

** Per 100 c.c. of serum.

†† June, 1944.

‡‡ January, 1945.

Her condition in the next few months can be described best by excerpts from her letters. On February 16, 1944 she wrote: "I'm getting along fine. Haven't felt bad at all. I feel much stronger and my appetite has come back. Also, my hair has grown so that

today I was able to get a 'permanent.' I went to see my doctor, and he said I was beginning to look like someone he used to know. That made me feel good." On March 16, 1944 she said: "I am still feeling better, but since I last wrote I have had trouble with my monthly periods. I had a normal period on the tenth of December and one on the seventh of January, but I completely skipped February and, so far, March. My hair is much thicker now and I have regained most of my strength. The above is the only thing that is not returning to normality." On April 19, 1944 she stated: "My periods have not recurred and for the past week I have had pains in my back similar to the ones I used to

TABLE 2. TREATMENT BEFORE AND AFTER REMOVAL OF ADRENAL CORTICAL TUMOR

Year, 1943	Desoxycorticosterone acetate, mg.	Cortical extract (Kendall), c.c.	Physiologic saline solution, c.c.
		Preoperative	
October 14	10		
15	10	50	1,000
		Postoperative	
15		50 45 30	1,000 1,000 1,000 U.R.*
16		30 30 30 30	1,000 1,000
17		10 10	1,000

* "U.R. solution" contains, in one liter, 2.8 Gm. of sodium chloride, 0.9 Gm. of potassium chloride and 30 Gm. of glucose.

have. However, as a whole, I am feeling better." On May 29, 1944 she wrote: "Everything seems to be going wrong again, so I am coming back to the clinic for a checkup."

When she returned on June 4, 1944, it was immediately apparent that she probably had a recurrence of the tumor. The face again was full and round; there was very extensive acne of the face, chest and back. The hirsutism had returned. The arms and legs were again thin, and there were marked cutaneous striations in the vicinity of the hip joints. The blood pressure was 140 systolic and 118 diastolic. Various laboratory studies were again conducted with essentially the same results as those obtained prior to operation. Urinary excretion of the 17-ketosteroids was 110 mg. per 24 hours, of which 60 per cent was of the beta type. Urinary estrogens were not determined.

Previous experience with cases of recurring carcinoma of the adrenal cortex had convinced us of the futility of roentgen therapy. The patient, however, was desperate and wanted something done. Although it seemed unlikely that further surgical treatment could accomplish anything, it was decided to explore the left adrenal region once more. Through a second posterolateral incision the left kidney was brought down, and at the upper pole a large mass could be felt. Superficially, the mass seemed well encapsulated, but as the operation proceeded, it was found that the recurrent tumor was very extensive and adherent to the surrounding structures. To remove the tumor it was necessary to remove the left kidney. The operation was very difficult to perform, and at its completion it was felt that all of the tumor had not been removed. The same preoperative and postoperative treatment that had been used at the first operation was again employed, and again the patient's postoperative convalescence proceeded uneventfully. From this operation the patient received very little benefit, although the urinary excretion of 17-ketosteroids was found on July 4 and July 10 to be 7.6 and 26.8 mg. respectively, in 24 hours.

Three months later, on September 14, 1944, the patient wrote to the effect that she was losing her hair again, had pains and weakness in the legs, "bloated stomach," no signs of a monthly period and a great deal of distress on urination. Roentgen therapy was advised and given without any benefit. Her condition became gradually worse, and she returned to the Clinic on November 23, 1944. The blood pressure was then 202 systolic and 128 diastolic. There was intense acne of the face, chest and back. Her voice was like that of an adolescent boy. There was a pronounced fatty dorsal hump. In fact, practically all of the initial signs and symptoms of her illness had recurred and had increased in severity. One of the striking symptoms was extreme muscular weakness, particularly of the legs, which were so weak that she had to be assisted in stepping up on an ordinary hospital scale.

The change for the worse was also evidenced by the results of laboratory examinations listed under date of January, 1945, in Table 1. The very low concentrations of the plasma potassium and chlorides and the high values for the plasma sodium and carbon dioxide combining power are particularly significant. The daily urinary excretion of 17-ketosteroids and estrogenic substances was also greatly increased. Roentgenograms of the chest showed a discrete rounded shadow about 1½ inches (3.8 cm.) in diameter, which was thought to represent a metastatic lesion. Marked osteoporosis of the vertebrae was noted in the roentgenograms of the spine. Cultures of the urine showed organisms which were thought to be *Proteus ammoniae*.

The patient failed rapidly. On December 15, 1944, she vomited a large amount of blood and passed blood by rectum. There was another massive hemorrhage from the gastro-intestinal tract on January 17, 1945. She died the next day.

Necropsy report.—The following final pathologic diagnoses were made: Cushing's syndrome due to carcinoma of the left adrenal with recurrence after adrenalectomy; extension of recurrent adrenal carcinoma to the spleen, pancreas, stomach and diaphragm; metastasis to the regional and aortic lymph nodes, liver and lungs; atrophy of the right adrenal gland; basophilic degeneration of the pituitary gland; failure of luteinization of graafian follicles; active duodenal ulcer with hemorrhage and nephrolithiasis on the right.

The following observations were made: In the left upper part of the peritoneal cavity and in the expected position of the left adrenal gland, there was a soft, yellow, partly hemorrhagic nodular mass measuring 15 by 13 by 8.5 cm. (Fig. 2). This was considered to be a recurrence of the adrenal tumor previously removed. There were metastatic nodules in the liver, similar in appearance to the main tumor. The largest of these measured 4.5 by 3.5 by 3 cm. Metastatic lesions were also present in the upper lobe of the

right lung and in both lobes of the left lung. These ranged in size from 0.3 to 5 cm. in diameter.

The right adrenal gland was atrophic and weighed 1.5 Gm. (normal weight 6.7 Gm.)

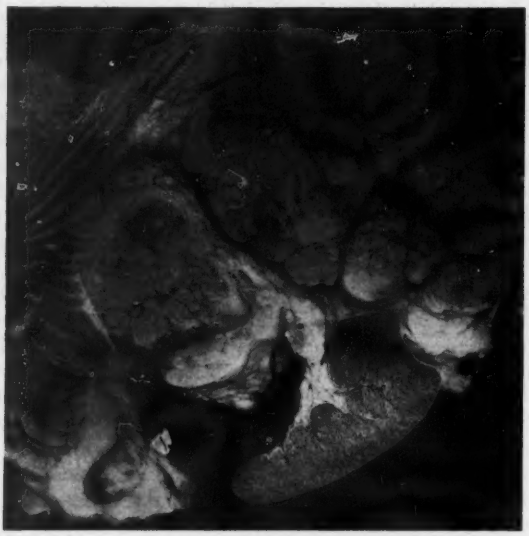

FIG. 2. Necropsy specimen. Recurrence of left adrenal cortical carcinoma with extension to spleen, stomach and tail of pancreas.

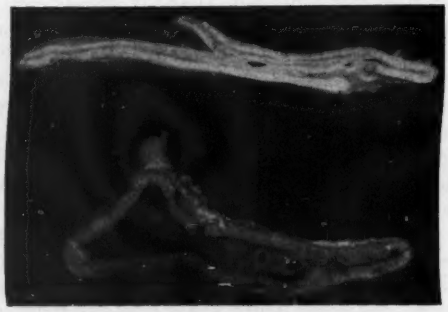

FIG. 3. Atrophic right adrenal gland (above) compared with a normal adrenal gland (below).

(Fig. 3). On inspection, the pituitary, thyroid and parathyroid glands appeared to be normal. The pancreas was grossly normal but its tail was adherent to the tumor. The ovaries appeared to be normal in size and appearance. The uterus measured 4 by 4 by 2.5 cm.

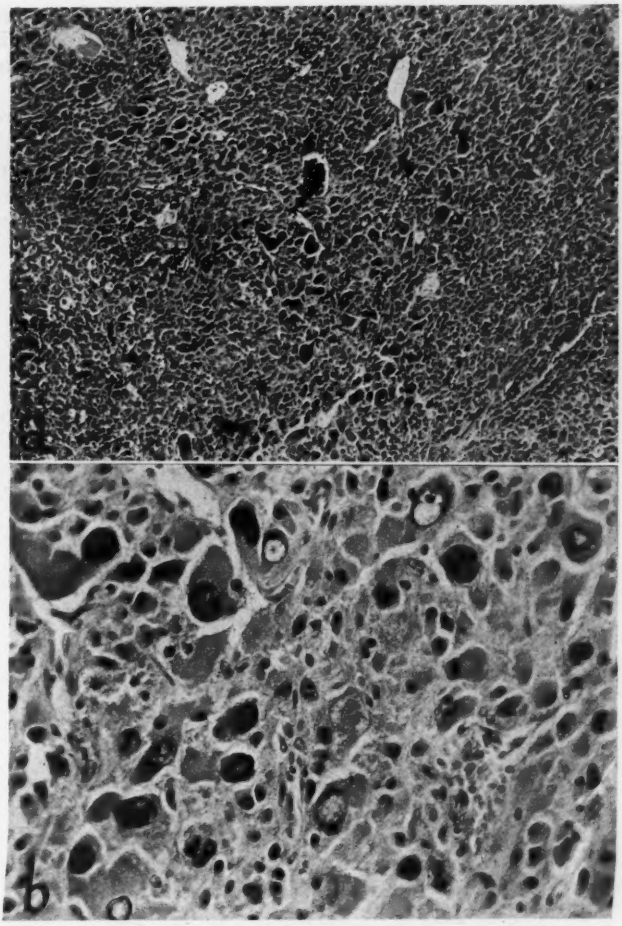

FIG. 4. Left adrenal cortical carcinoma showing pleomorphism and tumor giant cells (surgical specimen). *a.* ×57. *b.* ×220.

In the posterior wall of the duodenum, 2.5 cm. from the pylorus, there was an ulcer measuring 0.5 cm. in diameter which had eroded a small vessel and produced a fatal gastro-intestinal hemorrhage.

The right kidney weighed 280 Gm. In the pelvis there were three stones, measuring up to 0.8 cm. in diameter. The left kidney had been removed surgically.

The original left adrenal tumor, the recurrent tumor removed surgically, and the tumor found at necropsy were similar in appearance. All histologic sections of the left adrenal tumor and the recurrent lesions associated with it revealed an adenocarcinoma with cells resembling those of the adrenal cortex (Fig. 4*a* and *b*). The arrangement of these cells in cords and clusters with intervening blood vessels at times resembled the

zona glomerulosa and zona reticularis. More often, however, the cells were grouped in solid masses and bore no resemblance to any one zone of the adrenal cortex. The tumor was an adrenal adenocarcinoma graded 2 according to the method of Broders. Small areas of necrosis were present.

The individual cells revealed plemorphism. Many tumor giant cells were present. Foam cells were present in some areas. The average cell was 8 to 12 microns in diameter, but the diameter of the tumor giant cells ranged up to 40 or 50 microns. The nuclei were vesicular in many cells and in others, pyknotic. Their diameter in the average cell ranged from 6 to 8 microns, whereas in the tumor giant cells their diameter ranged up to

FIG. 5. Section of atrophic right adrenal gland. Entire thickness. of the gland is shown (×57).

20 microns. The cytoplasm was finely granular, at times vacuolated, but for the most part, acidophilic. A mitotic figure could be identified in every fourth or fifth high-power field.

Sections of the tumor stained with Ponceau-fuchsin revealed the cytoplasm of many cells taking the fuchsin stain. Fuchsinophil granules were present in only a few cells. The Masson trichrome stain likewise revealed only a few cells with granules which took the stain. Sections stained with Sudan III revealed moderate variation of the fat content within the cells of the tumor. In most areas only small droplets were present within the cytoplasm of the tumor cells, but in areas with early necrosis, the droplets were more numerous and of larger size.

FIG. 6. Section of anterior lobe of pituitary body showing absence of granules in basophile cells, as well as hyalinization and vacuolization of the basophiles (Crooke's changes) (×320).

Marked atrophy of all three cortical layers of the right adrenal had occurred; this was especially noticeable in the zona glomerulosa (Fig. 5). An occasional cell in the cortex revealed small fuchsinophil granules after staining by the Ponceau-fuchsin method. With the Sudan III stain a much greater content of fat could be seen in the cortical cells of the right adrenal than within the cells of the tumor.

The metastatic lesions were histologically identical in appearance with the primary tumor, but showed larger areas of necrosis and infarction. Invasion of lymph nodes was common. In the pulmonary veins, tumor thrombi were identified.

The duodenal ulcer was typical of a benign, active peptic ulcer and had penetrated into the pancreas. In the kidney there was occasional hyalinization of glomeruli. The medium-sized arteries revealed a mild degree of intimal hyalinization with slight medial hypertrophy. Some of the tubules had clear vacuolated cytoplasm and calcium deposits were present in many of the tubules of the cortex. The findings in the kidney were consistent with the vascular change of benign nephrosclerosis.

In the anterior lobe of the pituitary body there were two small chromophobe adenomas, the largest of which measured 1 mm. in diameter. The majority of the basophilic cells revealed the changes described by Crooke. These consisted of absence of granulations, vacuolization and homogenous hyalinization of the cytoplasm (Fig. 6).

In the thyroid, involution was the predominant feature. The acini were all lined with low cuboidal epithelium and were filled with colloid. The parathyroids were histologically normal. The predominant cell was the chief cell, and oxyphil cells were sparse.

There was some persistent thymic tissue which contained numerous large degenerating Hassall's corpuscles. The pancreas was normal in appearance throughout.

In the ovaries there was a decrease in the expected number of primordial follicles for a person of twenty-three years. The graafian follicles showed development of granulosa cells, but there was no evidence of luteinization. Some of the follicles showed fibrosis around granulosa cells giving the appearance that corpora albicantia were developing without the intermediary phase of luteinization (Fig. 7*a* and *b*).

The endometrium of the uterus appeared to be in the midproliferative phase. The glandular cells were composed of low columnar or cuboidal endothelium and contained no mucus. No tortuosity of the endometrial glands was observed. The endometrium showed no histologic evidence of progestational effect.

Grossly and microscopically, the brain appeared to be entirely normal. The paraventricular hypothalamic nuclei were studied carefully and showed none of the changes described by Heinbecker in cases of Cushing's disease.

PROCEDURE FOR METABOLIC STUDIES

Metabolic studies were conducted during seven periods, consisting of five or six days each. The dietary content of sodium and chloride was kept constant throughout the entire study. During the first two periods the potassium content of the diet was kept at a fairly low level. A further reduction in the intake of potassium was made in periods 3 and 4. In period 5 the intake of potassium was increased to a high level by the administration of potassium chloride. The patient was then operated on, and a left adrenal cortical tumor was removed. Twenty-four days were allowed for the patient to recover from the immediate effects of the operation. At the end of this time metabolic studies were resumed. The intake of potassium

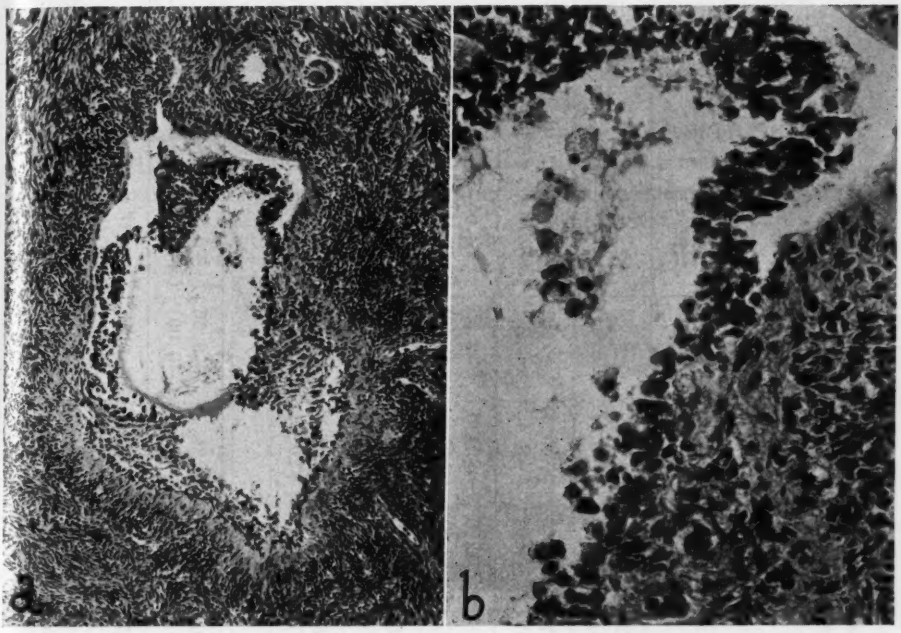

FIG. 7. Ovary showing graafian follicle with development of granulosa cells. The follicle shows beginning fibrosis, resembling formation of corpus albicans, with absence of luteinization. *a*. ×90. *b*. Details of granulosa cells (×350).

was again reduced to a very low level (periods 6 and 7) so that observations could be made and compared to the results obtained prior to the removal of the tumor.

Except for the changes made in the content of electrolytes, the diet was kept relatively constant throughout the seven periods. It supplied approximately 2,000 calories and contained approximately 200 Gm. of carbohydrate, 70 Gm. of protein and 100 Gm. of fat. The intake of fluid was also kept constant at 2,300 c.c. per day. In order to keep the intake of sodium and potassium at the desired level, food was cooked without salt and sodium chloride or potassium chloride was added. During periods 3 and 4, when the intake of potassium was greatly reduced, much of the food was cooked in parchment bags. The actual electrolyte content of the diet was determined by direct chemical analysis.

Analyses of the urine and stools were made for sodium, potassium, chloride, calcium and nitrogen. Urinary excretions of creatine and creatinine were also determined. At fairly regular intervals, blood was drawn for chemical studies.

TABLE 3. METABOLIC STUDIES IN A CASE OF CUSHING'S SYNDROME BEFORE AND AFTER REMOVAL OF MALIGNANT ADRENAL CORTICAL TUMOR

Period	Year, 1943	Days	Daily intake						Balance*						Body weight, Kg.
			mEq.			Gm.			mEq.			Gm.			
			Na	K	Cl	Ca	P	N	Na	K	Cl	Ca	P	N	
1	Sept. 15–20	6	95	62	98	0.78	1.01	10.6	+12.2 (+2.0)	−24.1 (−4.0)	+67.1 (+11.2)	−0.53 (−0.09)	−1.47 (−0.25)	−13.5 (−2.3)	55.7 56.2
2	Sept. 21–26	6	95	62	98	0.78	1.01	10.6	−41.7 (−7.0)	−25.6 (−4.3)	−50.5 (−8.4)	+1.51 (+0.25)	+0.58 (+0.10)	−10.0 (−1.7)	56.1
3	Sept. 27–Oct. 2	6	106	46	104	0.75	1.02	10.9	+59.5 (+9.9)	−83.0 (−13.8)	+34.0 (+5.7)	−2.55 (−0.43)	−2.65 (−0.44)	−10.2 (−1.7)	55.3
4	Oct. 3–7	5	108	47	105	0.74	1.03	10.8	+70.0 (+14.0)	+39.0 (+7.8)	+44.9 (+9.0)	−0.31 (−0.06)	−0.03 (−0.01)	−2.0 (−0.4)	56.3
5	Oct. 8–12	5	103	126	184	0.77	1.02	10.9	−48.6 (−9.7)	+164.0 (+32.8)	+104.3 (+20.9)	+1.68 +0.34)	+1.81 (+0.36)	+6.4 (+1.3)	56.3
	Oct. 15		Operation												
6	Nov. 8–13	6	104	42	102	0.70	0.95	10.1	+205.0 (+34.2)	+101.0 (+16.8)	+181.7 (+30.3)	−0.53 (−0.09)	+0.74 (+0.12)	+30.5 (+5.1)	55.7 56.1
7	Nov. 14–19	6	104	42	102	0.71	0.86	9.3	+302.5 (+50.4)	+98.0 (+16.3)	+292.0 (+48.7)	−0.13 −(0.02)	+1.16 (+0.19)	+23.5 (+3.9)	56.5

* Balances are given as total amount per period and, in parentheses, as average amount per day.

Venous blood for chemical determinations was drawn under oil into a tube containing purified heparin. The carbon dioxide content of the plasma was determined by the method of Van Slyke and Neill (1); pH by means of the glass electrode method as described by Dill, Daly and Forbes (2); urea by the manometric method (3); sodium by the method of Butler and Tuthill (4); potassium by Hartzler's modification of the method of Shohl and Bennett (5); calcium by precipitation as oxalate from ashed plasma;

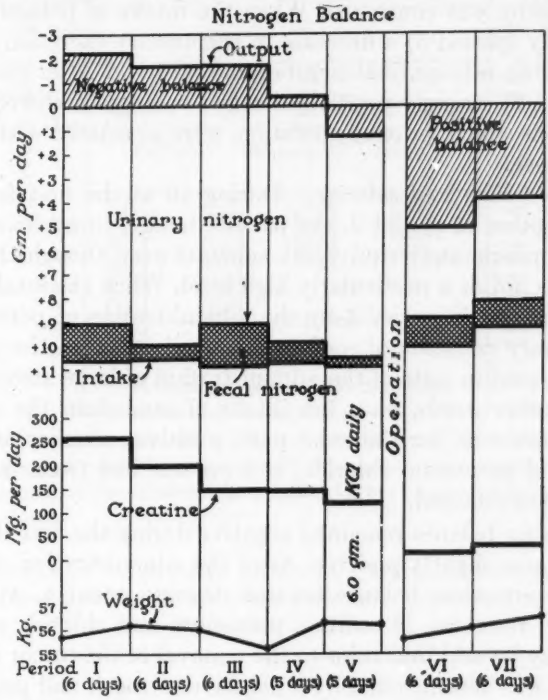

FIG. 8. Nitrogen balance, creatine nitrogen excretion and body weight before and after removal of malignant adrenal cortical tumor.

inorganic phosphorus by the method of Gomori (6); and chloride by a modification of the method of Keys (7).

In urine and in homogenized suspension of feces, chloride was determined by a modified Volhard-Harvey titration, and total nitrogen by the Kjeldahl method. Sodium, potassium, phosphorus and calcium were determined on ashed samples of these materials by the methods previously mentioned. Creatinine and creatine in urine were determined according to Folin (8), the latter by the open flask procedure.

RESULTS OF METABOLIC STUDIES

Results of the observations made are recorded in Tables 3, 4, 5, 6 and 7 and in Figures 8, 9 and 10.

Nitrogen.—Although the caloric value of the diet was adequate, the nitrogen balance was negative throughout the first four periods, during which the intake of potassium was at a low level (Table 3 and Fig. 8). The extent of the negative nitrogen balance tended to decrease as the low intake of potassium was continued. When the intake of potassium was increased sharply (period 5) a decrease in the urinary excretion of nitrogen followed and the nitrogen balance became positive. After the operation (periods 6 and 7) strongly positive nitrogen balances occurred under the same conditions which, prior to operation, were associated with a negative nitrogen balance.

Sodium, potassium and chloride.—During all of the first four periods, with the exception of period 2, the patient tended to retain sodium and chloride in approximately equivalent amounts even though the intake of these ions was not at a particularly high level. When the intake of potassium was increased in period 5 by the administration of potassium chloride, the urinary excretion of sodium increased, whereas the excretion of chloride decreased in spite of the administration of large amounts of chloride ion. In other words, on a low intake of potassium the sodium and chloride balance was, for the most part, positive; whereas after the administration of potassium chloride, sodium was lost from the body but chloride ion was retained.

The potassium balance remained negative during the first three periods and then became slightly positive. After the administration of potassium chloride, the potassium balance became strongly positive. After removal of the tumor, retention of sodium, potassium and chloride occurred. In general, it may be said that prior to the removal of the tumor a low intake of potassium was accompanied by a negative nitrogen and potassium balance and a retention of sodium. The chloride balance paralleled the sodium balance. When potassium chloride was administered, the body retained potassium and chloride but lost sodium.

The total balances of chloride, sodium, potassium and nitrogen before and after removal of the tumor are summarized in Figure 9.

Calcium and phosphorus.—In most respects, the balance of calcium and phosphorus paralleled the balance of nitrogen and potassium.

Data on intake and balance of sodium, potassium, chloride, calcium, phosphorus and nitrogen are given in Table 3. The balances of these substances before and after removal of the tumor are summarized in Table 4.

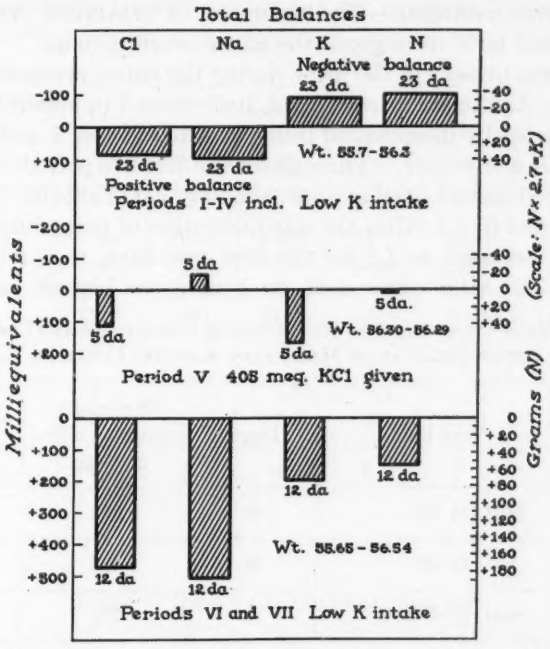

FIG. 9. Total balances of chloride, sodium, potassium and nitrogen before and after removal of malignant adrenal cortical tumor. The values represented by each column are the total balance of the substance in question for the number of days specified above or below the column.

TABLE 4. SUMMARY OF BALANCES IN A CASE OF CUSHING'S SYNDROME BEFORE AND AFTER REMOVAL OF MALIGNANT ADRENAL CORTICAL TUMOR

Period	Condition	Average daily balance					
		mEq.			Gm.		
		Na	K	Cl	Ca	P	N
1–4	Low preoperative intake of K	+ 4.4	− 4.1	+ 4.2	−0.08	−0.16	−1.55
5	High preoperative intake of K	− 9.7	+32.8	+20.9	+0.34	+0.36	+1.3
6–7	Low postoperative intake of K	+42.3	+16.6	+39.5	−0.06	+0.16	+4.5

Creatine and creatinine.—The excretion of creatinine remained at a rather constant level throughout the entire seven periods.

Creatine was present in the urine during the entire preoperative portion of the study. As the study continued, it decreased in amount. Postoperatively, it practically disappeared from the urine (Table 5 and Fig. 8).

Urinary pH and volume.—Throughout the first five periods the pH of the urine remained almost fixed at approximately 7.0 (Table 6). On four occasions it dropped to 6.5. After the administration of potassium chloride the urinary pH increased to 7.5 for the first two days, then fell back to its usual level, 7.0. After removal of the tumor, the highest value recorded

TABLE 5. AVERAGE DAILY URINARY EXCRETION OF CREATININE AND CREATINE BEFORE AND AFTER REMOVAL OF MALIGNANT ADRENAL CORTICAL TUMOR

Period	Year 1943	Days	Preformed creatinine nitrogen, mg.	Creatine nitrogen, mg.
1	Sept. 15–20	6	295	258
2	21–26	6	302	200
3	Sept. 27–Oct. 2	6	272	147
4	Oct. 3– 7	5	290	145
5	8-12	5	252	120
	Oct. 15 Operation			
6	Nov. 8–13	6	269	15
7	14–19	6	261	30

was 5.5 and during most of periods 6 and 7 the pH was reduced to 4.5 and remained relatively fixed at about that level.

Prior to operation, the daily urinary output varied from 1,275 c.c. to 2,250, averaging 1,700 c.c. After operation the average daily output was 980 c.c.; the range was from 400 to 1,600. Since the daily intake of fluid was kept at a constant level, the decreased output of urine which occurred postoperatively was indicative of retention of water. The administration of potassium chloride did not change the urinary output to any appreciable extent.

Plasma electrolytes.—The behavior of the plasma electrolytes is shown in Table 7 and Figure 10. It is apparent that during the periods in which the intake of potassium was kept at a low level, the concentration of the

TABLE 6. DATA ON THE VOLUME AND pH OF THE URINE

	Period						
	Preoperative					Postoperative	
	1	2	3	4	5	6	7
Volume							
Range, c.c.	1385–1990	1640–2250	1370–1875	1275–1690	1430–1880	805–1480	470–1160
Mean, c.c.	1712	1911	1555	1526	1619	1049	867
pH							
Range	6.5–7.0	6.5–7.0	6.5–7.5	6.5–7.0	7.0–7.5	4.5–5.0	4.5–5.5
Mean	6.8	6.7	6.9	6.9	7.2	4.6	4.7

TABLE 7. DATA ON PLASMA ELECTROLYTES

Period	Year, 1943	Days	mEq. per liter				Mg. per 100 c.c.		pH
			Na	K	Cl	CO_2	Ca	P	
1	September 15–20	6	133.9 136.0	4.4 5.2	94.5 96.7	32.0 33.0	9.8	1.8	
2	September 21–26	6	140.4	4.3	99.3	32.5	10.1	3.0	
3	September 27–October 2	6	142.6	4.2	94.5	34.7			
4	October 3–7	5	140.0	3.9	94.8	34.2	13.9	2.9	7.42
5	October 8–12	5	138.3	5.7	97.9	29.0	11.3	3.1	7.42 7.36
	October 15		Operation						
6	November 8–13	6	139.1 136.5	4.7 3.9	102.4 103.8	26.1 26.2	9.7 10.0	4.8 5.3	
7	November 14–19	6	140.0	3.9	104.4	25.3	9.2	5.3	

plasma potassium decreased progressively and was, for the most part, accompanied by an increase in the concentration of the plasma sodium. Simultaneously there occurred a slight rise in the carbon dioxide content of the blood plasma and possibly a slight decline in the level of the chloride. These tendencies were corrected by the administration of potassium chloride. In addition, when the intake of potassium was increased the pH of the blood decreased. These data taken alone are perhaps not of much sig-

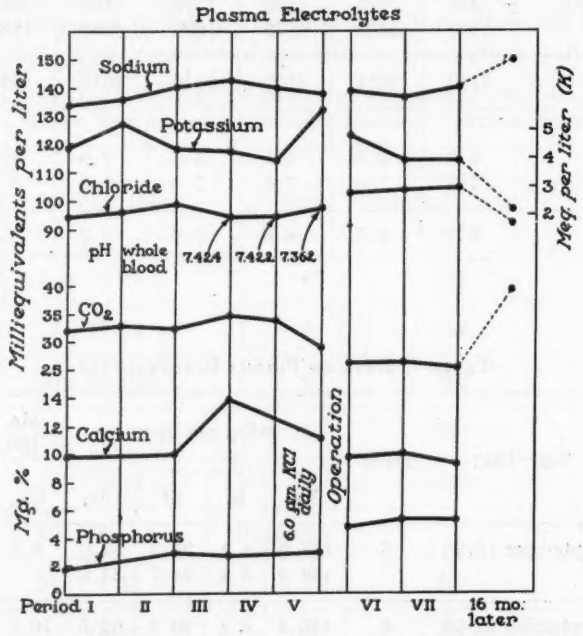

FIG. 10. Values for the plasma electrolytes and pH of whole blood during the several periods of study and after final recurrence of malignant adrenal cortical tumor.

nificance; but when they are compared with those we had obtained in previous studies on other patients (9, 10) and with those on the chemical status of the blood when the patient was again examined in January, 1945, their importance increases. It will be noticed that at the time the patient was studied in January, 1945, when the tumor had recurred, the hypokalemic hypochloremic alkalosis was thoroughly established and was accompanied by high values for the plasma sodium.

Urinary steroids.—Preoperative determinations of the urinary 17-ketosteroids gave values of 136, 125 and 132 mg. in 24 hours of which 71.5 per cent were 3(β)-hydroxy-17-ketosteroids. On the fourth day after oper-

ation these values fell abruptly to 2.4 mg. This level of excretion was maintained, with some fluctuations, until the patient was dismissed approximately six weeks later. Four months after dismissal, the quantity of 17-ketosteroids excreted was 50 mg. in 24 hours and in another two months it had increased to 107 mg. of which 60 per cent were 3(β)-hydroxy-17-ketosteroids. Immediately after the second operation the amount of 17-ketosteroids excreted was 7.6 mg. but in six days it had increased to 26.8 mg. Six months later it was 314 mg. in 24 hours.

One preoperative bio-assay for estrogen gave a value of 9,400 rat units in 24 hours. The values for the first seven days after operation were 1,700, 1,000, 100, 170, 120, 80 and 43 rat units respectively. A month later two normal values of 30 rat units were obtained. Six months after the second operation the quantity of estrogen excreted was 5,000 rat units in 24 hours.

The procedures for the isolation of the steroids from the urine in this case and the substances isolated have been described elsewhere (11) (case 5). The neutral fraction obtained from 43 liters of urine collected during a period of twenty-six days prior to operation weighed 9.3 Gm. Fractionation resulted in the isolation of 2,190 mg. of dehydroisoandrosterone, 192 mg. of etiocholan-3(α)-ol-17-one, 49 mg. of androsterone, 11 mg. of androstane-3(α), 11(β)-diol-17-one, 107 mg. of pregnane-3(α), 20(α)-diol, 55 mg. of Δ^5-androstene-3(β), 17(α)-diol, 10 mg. of Δ^5-androstene-3(β),16(β),17(α)-triol and 20 mg. of a substance with the empirical formula $C_{19}H_{32}O_2$.

The phenolic fraction contained 88,800 rat units of estrogen which was the equivalent of 177 mg. of estrone by the procedure used. Fractionation and isolation gave 62 mg. of estrone which represented 35 per cent of the total estrogenic activity. Estradiol and estriol could not be isolated.

COMMENT ON PATHOLOGIC ANATOMY.

Among the anatomic abnormalities noted at necropsy, the following perhaps are the most significant: 1) atrophy of the contralateral adrenal cortex, 2) Crooke's changes in the basophilic cells of the pituitary body, 3) chromophobe adenomas of the pituitary body, nephrolithiasis, nephrosclerosis, the absence of changes in the hypothalamus, and the failure of the graafian follicles to luteinize.

Contralateral adrenal cortical atrophy—Removal of benign or malignant functioning neoplasms of many of the endocrine glands is frequently followed by easily recognized symptoms suggesting reversible atrophy (disuse?) of the residual nontumorous endocrine tissue from which the neoplasm arose. The disastrous postoperative consequences of this atrophy in the case of adrenal cortical tumors have been repeatedly emphasized. Cahill (12) has expressed the opinion that acute postoperative adrenal

cortical insufficiency of severe degree is particularly likely to occur in cases of Cushing's syndrome, in contrast to its relatively infrequent occurrence in cases of adrenogenital syndrome. In our experience this generality seems to be true, although we still think it is advisable to treat preoperatively all patients having adrenal cortical tumor on the assumption that acute adrenal insufficiency may occur postoperatively.

"Crooke's changes" in the pituitary body.—Hyalinization of the basophilic cells of the pituitary body occurs regularly in cases of Cushing's syndrome and rarely in any other condition (13). This association holds true regardless of the presence or absence of recognizable adrenal cortical lesions. Crooke concluded that these changes were the anatomic accompaniment of a functional disturbance in the basophilic cells and that this disturbance was of fundamental significance in the etiology and pathogenesis of Cushing's syndrome. In a previous article (14) one of us (E. J. K.) presented a critical analysis of the significance of Crooke's phenomena and suggested that the changes in the basophilic cells were retrograde and perhaps degenerative in character. At this time we are merely calling attention to the difficulties in applying Crooke's concept to the case we have reported. In this case, if Crooke's contention and its implications are accepted, one almost has to conclude that an agent continually secreted by the hyalinized basophilic cells stimulated one adrenal cortex so intensely that neoplastic changes occurred, and simultaneously either caused or failed to prevent atrophic changes in the contralateral gland. One might explain the atrophy of the contralateral gland by assuming that the tumor grew because of the stimulus of pituitary adrenotropin, then became capable of hyperfunction independent of any tropic influence by the pituitary and finally inhibited the production of adrenotropin. On the other hand, rather than postulate excessive production of a pituitary tropic principle which was directly or indirectly responsible for the adrenal neoplasm and then try to reconcile the contralateral adrenal cortical atrophy with the postulate, it seems to us more reasonable to assume that the adrenal neoplasm grew and functioned *sui generis*, and in so doing caused the contralateral atrophy either directly or indirectly by inhibiting the production of pituitary adrenotropic material.

Associated chromophobe adenoma of the pituitary body.—Adventitious adenomas have been observed repeatedly in almost all of the endocrine organs, and the two tiny chromophobe adenomas that were found in our case possibly can be regarded as examples. On the other hand, it has been shown repeatedly in animals that the continuous administration of estrogens is followed by the appearance of adenomatous proliferation of chromophobe cells of the anterior lobe of the pituitary body (15, 16, 17). These changes occur in a species, namely, mice and rats, in which spontaneously

occurring hypophyseal tumors rarely are encountered. These observations, when taken in conjunction with the high urinary estrogenic content observed in our patient, make one suspect that the two chromophobe adenomas were not incidental but may have occurred as the result of the pathologic production of large amounts of estrogenic material by the adrenal tumor.

Nephrosclerosis and nephrolithiasis.—Renal calculi and renal colic are relatively frequent complications of Cushing's syndrome. This association is probably intimately related to the negative calcium balance and the consequential osteoporosis which is often an integral part of the syndrome. Albright (18) has pointed out that the negative calcium balance is in turn a manifestation of a negative nitrogen balance. According to him, negative nitrogen balance results in a loss of the protein matrix of bone, and this in turn is associated with a negative calcium balance, osteoporosis, calciuria and, in some instances, renal calculi and calcinosis.

Hypertension, one of the cardinal symptoms of Cushing's syndrome, might be followed by nephrosclerosis; conversely, nephrosclerosis conceivably might induce hypertension. (Selye (19) has produced severe experimental nephrosclerosis by the administration of desoxycorticosterone acetate.) Either of the two possible interpretations mentioned above might be applicable in our case.

Absence of follicular luteinization.—The histologic appearance of the endometrium was in keeping with, and reflected the histopathologic characteristics of the ovaries. The complexity of the endocrine disturbance present makes interpretation of the ovarian pathologic process practically impossible. Among the factors which would have to be considered are the simultaneous overproduction of both androgenic and estrogenic substances by the neoplasm and their retrograde effect on the pituitary body and its production of gonadotropic substances.

Hypothalamic lesions.—Degeneration of the hypothalamic nuclei and pathways has been noted in cases of Cushing's syndrome not associated with adrenal cortical tumor, and the possibility has been suggested that these degenerative lesions have etiologic importance (20). Our observations throw no light on this question.

COMMENT ON METABOLIC STUDIES

Since we are dealing in this report with only one case, the scope of our discussion concerning the alterations of metabolism in cases of Cushing's syndrome must be restricted. Certain features seem worthy of comment.

Nitrogen.—Prior to operation, the patient had a negative nitrogen balance, which was not great but which was, nevertheless, definite. Loss of nitrogen tended to decrease during the latter periods before operation.

This fact, in conjunction with Albright's (18) observations on the nitrogen balance in Cushing's syndrome, make one suspect that loss of tissue may occur intermittently,[1] or may first progress to a maximum, then decrease and finally cease entirely. Clinically, evidence indicative of loss of nitrogen-containing tissues is impressive in nearly all patients having Cushing's syndrome. After removal of the adrenal tumor there was a very strongly positive nitrogen balance. This dramatic postoperative retention of nitrogen is perhaps a better index of the antecedent loss of tissue than the preoperative balances indicate.

Administration of potassium chloride preoperatively was associated with a positive nitrogen balance. Usually loss of protein from the body is associated with loss of potassium; and conversely, synthesis of protein by the body is accompanied by retention of potassium. Is intracellular retention of potassium necessarily accompanied by retention of nitrogen? Can synthesis of protein take place in the body despite inadequate intake of potassium? Is the intake of potassium or the forced loss of potassium such as might occur in Cushing's syndrome or possibly in other disorders, ever a critical factor in the anabolism or catabolism of protein? We think these questions are worth consideration.

Our data throw no light on the interesting possibility postulated by Albright (18) that the adrenal steroids influencing carbohydrate and protein metabolism are antianabolic rather than catabolic in their action.

Creatine.—Creatine was found in the urine during the five preoperative periods. After removal of the adrenal tumor it practically disappeared. Creatinuria commonly occurs in association with wasting of muscle. In this case it can be regarded either as a manifestation of muscular wastage or as a specific effect of some substance produced by the adrenal tumor, or both. The decreased excretion of creatine in periods 3, 4 and 5 as compared with periods 1 and 2 is probably explainable on the basis of a decreased intake of creatine due to removal of this substance by dialysis during the cooking of meat in parchment bags for the very low potassium diet which was employed in periods 3, 4 and 5.

Sodium and potassium balances.—In general, sodium and chloride were retained preoperatively, whereas potassium was lost from the body. These are the phenomena that one might expect to occur if the adrenal cortical tumor was the source of excessive amounts of substances having properties resembling those of desoxycorticosterone acetate. They are also in keeping

[1] Fluctuations in intensity of the symptoms have occurred in several of our cases of Cushing's syndrome. In one, a complete remission occurred without treatment and lasted for about three months. The case reported by Josephson (21) is of particular interest in this respect. A comparable case has been observed by Albright (22).

with the tendency to become edematous, which these patients frequently exhibit.

Administration of potassium was followed by increased excretion of sodium and retention of potassium. Retention of potassium, however, exceeded loss of sodium. After operation, retention of sodium, chloride and potassium occurred. The retention of sodium and chloride was at first surprising and apparently not in keeping with preoperative trends. However, comparable retention of sodium and chloride has been observed in other conditions when negative nitrogen balances are suddenly corrected and rapid synthesis of protein presumably occurs. For example, emaciated diabetics, victims of starvation and patients having anorexia nervosa frequently become edematous following institution of appropriate therapy. These are other examples of the intimate relationship between the metabolism of nitrogen and electrolytes.

Total metabolism.—In spite of marked retention of sodium, chloride, potassium, water and nitrogen the patient did not gain any significant amount of weight postoperatively. By the usual methods of calculation she should have gained about 5 Kg. We have given a great deal of thought to this paradox and offer this tentative suggestion. Patients having Cushing's syndrome become fat while they lose muscle. The net result is usually gain in weight, although loss of weight may occur. In either event, changes in weight throw little light on what is taking place physiologically. If the pathologic physiology is corrected, gain in muscle and loss of fat should occur simultaneously. Here again, the scales may be misleading for if the two tendencies are approximately equal, weight would remain unchanged, although the appearance of the patient would change materially. On the other hand, if the weight of the fat lost was greater than the weight of the muscle gained, the net result would be a decrease in body weight. Clinically this latter phenomenon has been observed repeatedly after removal of adrenal cortical tumors. A comparable phenomenon frequently occurs during adolescence. The child eats ravenously, gains in height, loses its childish contours, becomes gangly and gains little weight, much to the concern of the apprehensive parent. Unfortunately, we did not make serial determinations of the basal metabolic rate or determine insensible loss of water. Postoperatively, there was a significant increase in the basal metabolic rate, from 0 to 15 per cent, and a decrease in the respiratory quotient, from 0.83 to 0.74. Both of these observations are in keeping with the explanation we have suggested.

Urinary pH.—We have been impressed by the frequency with which patients having Cushing's syndrome excrete urine that is alkaline to litmus and also by the frequency with which infections of the urinary tract occur. The relatively high pH of the urine prior to operation and the sharp de-

crease in the pH which occurred postoperatively might well be reflections of the disordered metabolism of sodium and potassium and the tendency for alkalosis to develop.

Plasma electrolytes.—In previous studies it was shown that the administration of potassium chloride corrected the hypochloremic, hypokalemic alkalosis of Cushing's syndrome. It was logical to infer that restriction of potassium would make it worse. Our data tend to support this inference. When first seen, the patient had a mild alkalosis. Restriction of potassium was accompanied by a slight depression of the concentrations of plasma potassium and chloride and slight increases of the values for plasma sodium and carbon dioxide. This tendency was corrected by the administration of potassium chloride. After removal of the adrenal tumor the plasma electrolyte pattern was normal even though the intake of potassium was restricted. Sixteen months later, when the tumor had recurred, the intensity of the alkalosis was as severe as we have seen in cases of Cushing's syndrome.

Urinary steroids.—In most respects, the quantity and variety of steroids isolated from the urine resembled those that have been found in similar cases. Excretion of 17-ketosteroids was increased and there was a high concentration of the beta fraction, chiefly because of the presence of a large amount of dehydroisoandrosterone. The large amount of estrogenic material that was isolated is of considerable interest. Estrogenic substances have been found in a few cases of adrenal cortical tumor and sometimes, but more often not, in conjunction with feminizing syndromes. The simultaneous excretion of estrogens and steroids that have androgenic properties is unusual but not surprising. It again emphasizes the protean functional capacities of adrenal cortical neoplasms. Nearly thirty adrenal steroids have been isolated from the adrenal glands of animals. Some of these influence the metabolism of salt and water, others influence the metabolism of carbohydrate and protein, while 11-dehydrocorticosterone (compound A) or others may influence metabolism of fat and still others have androgenic, estrogenic or progestational properties. The variety of clinical pictures, the quantitative and qualitative variation in the urinary steroids and the absence of histologic homogeneity in the tumor all tend to support the thesis that these neoplasms can produce in excessive amounts almost any one or all of normal or even abnormal adrenal steroids.[2]

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[2] For a more detailed discussion of the mechanism by which the pathologic physiology of Cushing's syndrome might be produced by an excess of adrenal steroids, the reader is referred to articles by Kenyon (23), Albright (18), and Kepler and associates (24).

COMMENT ON THE PATHOGENESIS OF CUSHING'S DISEASE AND CUSHING'S SYNDROME.

Although we are reporting a case of adrenal cortical tumor associated with Cushing's syndrome, one of us (E. J. K.) is taking this opportunity to discuss the pathogenesis of Cushing's disease. Before proceeding further, a few historical remarks are pertinent.

Most of the early efforts to account for the clinical picture of Cushing's disease emphasized the role of the anterior lobe of the pituitary body. It was assumed that adenomatous basophilic cells produced excessive amounts of various pituitary hormones and that these in turn were responsible for the clinical symptoms, either by stimulating other members of the endocrine system or by acting on other loci in the body. This view was strengthened when it was shown that roentgen therapy to the pituitary body sometimes was followed by a remission of symptoms. Later observations discredited the part played by the adenomatous basophilic cells and incriminated the cells described by Crooke.

When it became apparent that the essential features of the syndrome were not restricted to cases of basophilic tumors of the pituitary body but could occur in association with any one of several pathologic lesions, particularly adrenal cortical hyperplasia and adrenal cortical tumor, another school of thought developed. This school emphasized the importance of the adrenal cortices. Some of its members maintained that the disease began as a primary disorder of the adrenal cortices. Others held the view that the primary disturbance began elsewhere, usually in the pituitary body but that most, if not all, of the endocrine symptoms in all cases of Cushing's syndrome were an expression of disturbances, hyperfunctioning in character, of the adrenal cortices. Their position was greatly strengthened after it was shown that when Cushing's syndrome was associated with adrenal cortical tumor, surgical removal of the tumor was followed by a prompt and dramatic remission of the endocrine symptoms. There was, nevertheless, one very formidable objection to their theory. Since adrenal cortical insufficiency was characterized by disturbances in the metabolism of electrolytes, why did not the hyperfunctioning adrenal cortical lesions also disturb the electrolyte pattern of the blood? In due time, this objection was answered by the appearance of a number of cases of Cushing's syndrome in which there were associated electrolytic abnormalities. True, the abnormal electrolyte pattern was not exactly the antithesis of that which was found in Addison's disease; but it was sufficiently antithetic in character to make one suspect that it was the result of excessive adrenal cortical activity. The first group of these examples consisted almost entirely of cases of adrenal cortical "hyperplasia." There were in the group,

however, some cases in which the adrenal cortices were reported to be grossly and microscopically normal; consequently, it could be argued that the abnormal electrolyte pattern of the blood was not necessarily the result of adrenal cortical hyperfunction. Subsequently, cases were discovered in which the hyperfunctioning adrenal lesion was proved to be an adrenal cortical tumor.[3] These cases of adrenal cortical tumor are particularly important in that they established beyond any reasonable doubt that the disturbance in electrolytes was of adrenal origin. In the case we have reported in this paper, the evidence is particularly convincing because the chemical condition of the blood each time became normal after the two removals of the tumor and each time became abnormal again after the two recurrences. In a sense, then, these cases of adrenal cortical tumor can be regarded as a missing link, so that now one can state with finality that all of the endocrine symptoms which thus far have been reported to occur in cases of Cushing's disease have also been found in cases of adrenal cortical tumor.

If adrenal cortical hyperfunction can account for all of the symptoms of Cushing's disease, what part does the anterior part of the pituitary body play in the pathogenesis of this condition? At least two possibilities come to mind. First, one can assume that for some unknown reason basophilic hyperfunction occurs. This hyperfunction is characterized by the overproduction of an adrenotropic agent which stimulates the adrenal cortices. The anatomic response to this stimulation is adrenal cortical hyperplasia or neoplasia; the functional response is adrenal cortical hyperfunction. Note that this assumption seems to imply that the basophilic hyperfunction is limited in scope and restricted in action to the overproduction of adrenotropic substance alone, for the reason that if more than one type of tropic agent were produced some endocrine symptoms would persist after the hyperfunctioning adrenal cortical neoplasm had been removed. This implication in turn results in either one of two others: (a) normal basophilic cells are able to make several hormones but in Cushing's syndrome their function is disturbed so that they make only one, an adrenotropic substance, in excess; or (b) normal basophilic cells can make only one tropic agent and of this one, in Cushing's syndrome, they make too much. Neither of the alternatives is particularly satisfying. One dislikes the first because in what is probably an analogous situation, eosinophilic pituitary hyperfunction (acromegaly) usually seems to be associated with the overproduction of several pituitary principles such as the growth hormone, thyrotropic hormone and the diabetogenic hormone. These, in turn, are

[3] One is the case which we have reported herein; another we observed but did not report. Albright (22) encountered a third case and others are mentioned in the literature. (23, 25).

responsible for the continued growth, the thyroid enlargement and the diabetes that go with this condition. One dislikes the second alternative because it seems to relegate the production of all the hormones of the anterior part of the pituitary body, except the adrenotropic, to the eosinophilic cells.

With regard to the second possibility, as to the part played by the anterior lobe of the pituitary body, one can assume that for some unknown reason the adrenal cortices become hyperplastic or neoplastic and hyperfunction; but that this reaction cannot take place unless the anterior part of the pituitary body is reasonably intact functionally. According to this view the pituitary body does not instigate the disorder but merely provides a set of conditions without which the real etiologic agent cannot be effective. This distinction between a necessary condition and a prime etiologic factor is a bit slippery, but it can be exemplified easily. Before the micro-organism responsible for pulmonary tuberculosis was discovered, inanition, bad air and overcrowding seemed to be the "cause" of tuberculosis because when these factors were eliminated the epidemiologic characteristics of the disease could be practically eliminated from a community. Now it is apparent that these "predisposing" factors do not "cause," but merely provide conditions which are necessary or favorable for the dissemination of the tubercle bacilli and its growth and multiplication in the affected host. Likewise, in the case of Cushing's syndrome (or for that matter, diabetes mellitus and exophthalmic goiter) there is the logical possibility that the anterior lobe of the pituitary body merely provides a set of circumstances that are necessary for the development of the syndrome. If this were the case, therapeutic radiation or any other form of therapy which would alter or reduce the function of the anterior part of the pituitary might be followed by remission or favorable alteration of the symptoms.

One of the foundations of the pituitary theory of Cushing's syndrome is the frequency with which basophilic tumors of the pituitary body are found. This high incidence cannot be dismissed lightly and requires for its interpretation something more than a philosophical "brushoff." Cushing (26) reasoned that since eosinophilic tumors hyperfunction and cause acromegaly, basophilic tumors might also hyperfunction and cause "basophilism." His concept was contested on two grounds: first, many of the tumors were so small that they escaped detection unless special search was made for them; second, basophilic tumors often were found in cases in which none of the symptoms that he had described was present. Neither of these objections is valid. Cushing disposed of the first, for the most part, by calling attention to the fact that size *per se* of an adenoma is no indication of its functional potentialities. This is apparent in the case of tiny hyperfunc-

tioning parathyroid tumors or islet cell tumors of the pancreas. The second objection, based on frequency of occurrence, likewise carries little weight, since it is now well known that, generally speaking, symptomatically silent endocrine tumors are found at necropsy much more frequently than are tumors which have been proved to cause symptoms.

If the objections based upon size of a tumor and the frequency with which nonfunctional tumors occur can be dismissed as being insignificant, what further objections, other than those that can be raised from purely clinical considerations, are there to the original pituitary theory and its various modifications? There is one objection, and that one is largely inferential in character; namely, that development of basophilic tumors, as well as the Crooke's changes, might be a retrograde phenomenon. Elsewhere in this paper, attention was called to the fact that chromophobe tumors of the anterior lobe of the pituitary body have been produced in animals by the prolonged administration of estrogenic substances. If this is the case, it is not illogical to suspect that administration of some of the adrenal steroids might be followed by the appearance of basophilic tumors. There is some evidence, not conclusive to be sure, that bears on this point. Woolley (27) in a personal communication to one of us (E. J. K.) has said that the experimental production of tumors of the adrenal cortex has been followed by the appearance of basophilic adenomatous changes in the anterior lobe of the pituitary body. He, however, was not altogether satisfied with the cytologic examinations that were employed. Furthermore, the tumors of the adrenal were induced in gonadectomized mice, and consequently, there was the possibility that the adrenal tumors might have been induced by the well-known changes in the function of the anterior lobe that occur after the gonads have been removed.

The pathogenesis of Cushing's disease involves more than an intellectual challenge. This disease, in our experience, is one of the most difficult and often one of the most unsatisfactory of all endocrinopathies to treat, and all types of treatment employed or recommended have been bases entirely on hypotheses. Even when the syndrome is associated with an adrenal cortical tumor, the satisfaction that goes with brilliant immediate cure is too often short-lived because of a fatal recurrence of the tumor or metastasis to the liver or lungs. If these disorders actually begin as a disturbance of the adrenal cortices, it seems illogical and unnecessary to subject the patient to roentgen therapy to the anterior lobe of the pituitary body, particularly when the therapeutic results are often disappointing and uncertain. If the fundamental anatomic site of the disorder is in the pituitary body, therapy should be directed toward depressing its adrenotropic function by any means now available or yet to be discovered, not only in cases of adrenal cortical hyperplasia, but even in cases of adrenal cortical tumor after the tumor has been removed.

At the time of this writing in 1947, the question of the adrenal versus the pituitary theory of Cushing's syndrome is still open. The problem might be approached experimentally from at least two angles. Patients with Cushing's syndrome might be studied for the content of adrenotropic substance in the blood and urine after thoroughly reliable methods become available. Some efforts along this line have been made. There is still some room for skepticism, however, regarding the conclusions which are based on the methods now employed. If the condition is characterized regularly by a high concentration of adrenotropic substance in the blood and urine one might well conclude that among the endocrine organs the anterior lobe of the pituitary body is primarily at fault. On the other hand, if the administration of adrenal steroids, particularly those steroids which affect the metabolism of protein and carbohydrate, such as compound E (11-dehydro-17-hydroxycorticosterone), should be followed in suitable experimental animals by the appearance of Crooke's changes or basophilic tumors or both, in the anterior lobe of the pituitary body, it would seem that the adrenal cortices are primarily at fault and that the pituitary abnormalities are secondary or at least incidental and could be ignored therapeutically.

SUMMARY

A case of adrenal cortical tumor has been reported. The patient presented the clinical features of Cushing's syndrome, including hypertension and hypokalemic, hypochloremic alkalosis. Urinary excretion of both 17-ketosteroids and estrogenic substances was increased to abnormally high levels. The beta fraction of the urinary 17-ketosteroids was found to be increased. Remission of symptoms followed the removal of the tumor. Recurrence of the tumor was accompanied by the reappearance of the clinical picture. Removal of the recurrent tumor was followed by a temporary and incomplete remission of symptoms. Recurrence of the tumor and the appearance of metastatic lesions in the lungs were associated with the symptoms that were present prior to the removal of the tumor. Roentgen therapy proved to be ineffective. At necropsy the following significant observations were made: the anterior lobe of the pituitary body contained two small chromophobe tumors; Crooke's changes were found to be present in the basophilic cells of the anterior lobe; the hypothalamus appeared normal; the tumor had recurred locally and had metastasized to the lungs; the contralateral adrenal cortex was found to be atrophic; although the patient had osteoporosis, the parathyroid bodies appeared to be normal; and the endometrium presented evidence of an apparent absence of progestational effects. The immediate cause of death was a bleeding duodenal ulcer.

A large quantity of urine was collected before operation and various steroidal compounds were isolated and identified. These included dehydroisoandrosterone; etiocholan-3(α)-ol-17-one; androsterone; androstane-

3(α), 11(β)-diol-17-one; pregnane-3(α),20(α)-diol; Δ^5-androstene-3(β),17(α)-diol; Δ^5-androstene-3(β), 16(β), 17(α)-triol; estrone and a substance with the empirical formula $C_{19}H_{32}O_2$.

Metabolic studies were conducted before the first operation and shortly thereafter. Under the conditions employed, the over-all nitrogen balance before operation was negative. Creatine was present in the urine. Potassium was lost from the body and sodium was retained when the potassium content of the diet was kept at a low level. Administration of potassium chloride tended to correct the alkalosis, the negative balances for potassium and nitrogen and the positive balance for sodium. After removal of the tumor, nitrogen, sodium, potassium and chloride all were retained in large amounts, and creatinuria practically disappeared. The amount and pH of the urine decreased postoperatively. The metabolic data suggested that the functioning adrenal tumor caused loss of muscle and deposition of fat and that its removal was followed by loss of fat and deposition of muscle.

The pituitary versus the adrenal theory of the pathogenesis of Cushing's disease has been discussed, and clinical and experimental methods which might eventually prove helpful in establishing or refuting one or the other of these theories have been suggested.

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THYROTOXICOSIS FACTITIA (ALIMENTARY THYROTOXICOSIS)

ITS DIFFERENTIATION FROM SPONTANEOUS THYROTOXICOSIS WITH THE AID OF RADIOACTIVE IODINE

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THE clinical picture of self-induced hyperthyroidism may be indistinguishable from that of spontaneous thyrotoxicosis but the history usually makes the differential diagnosis possible. However, some investigators have pointed out clinical symptoms by which they feel one should be able to differentiate between the two conditions. Falta (1) stated that eye signs never were present in simple hyperfunction of the thyroid. Several later investigators have been of the same opinion. Nevertheless patients with alimentary hyperthyroidism may present slight eye signs of the type seen in spontaneous thyrotoxicosis, particularly if they have previously had true Graves' disease. On the other hand, patients with endogenous thyrotoxicosis may show minimal or no eye signs. The eye signs are, therefore, not dependable in the differential diagnosis.

Enlargement of the thyroid gland is usually observed in endogenous thyrotoxicosis, but occasionally palpation of the thyroid reveals no enlargement. On the other hand, patients with induced hyperthyroidism, in whom one would expect a gland of normal size, may have an enlargement due to a nontoxic goiter. Therefore one cannot make the differential diagnosis by the physical examination. In most cases of alimentary thyrotoxicosis, however, the diagnosis can be made from the history of an excessive intake of thyroid, usually in an effort to reduce weight. With cessation of the thyroid medication the symptoms will subside in a few weeks and the diagnosis can be confirmed.

One of the greatest difficulties in the differential diagnosis between these two conditions is encountered with patients having psychiatric problems, who will not admit that they are taking thyroid.

Perkin, McFarland and Hurxthal (2) and Hurxthal (3) have studied a number of patients with secretly self-induced hyperthyroidism and found that urinary iodine determinations were of great help in establishing the

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diagnosis. In normal individuals the urinary output of iodine amounted to approximately 100 to 150 micrograms per day and in patients with spontaneous hyperthyroidism the excretion was approximately 250 micrograms per day. However, if the thyrotoxicosis was induced by thyroid medication of 0.3 Gm. or more of desiccated thyroid daily, the urinary output of iodine was still higher.

Accurate diagnosis of thyroid disorders has been greatly facilitated by the use of serum protein-bound iodine determinations as an index of circulating thyroid hormone and of tracer doses of radioactive iodine as an index of the avidity of the thyroid gland for iodide. The protein-bound iodine of serum is almost invariably elevated in thyrotoxicosis [Salter, Bassett and Sappington (4); Winkler, Riggs, Thompson and Man (5)].

In connection with the problem of differential diagnosis it is especially interesting to note that Riggs, Man and Winkler (6) found that the administration of large quantities of dried thyroid to normal subjects produced a clinical picture of hyperthyroidism with most of the signs and symptoms of spontaneous hyperthyroidism, including an elevation of both serum-precipitable iodine and basal metabolic rate.

Tracer doses of radioactive iodine can be used for diagnostic purposes as was first demonstrated by Hamilton and Soley (7). Keating, Power, Berkson and Haines (8) found that the urinary excretion of tracer doses of radioactive iodine was less than normal in hyperthyroid patients. In a clinical study by McArthur, Rawson, Fluharty and Means (9), it was demonstrated that a low urinary excretion of radioactive iodine was helpful in establishing the diagnosis of Graves' disease, and that a high excretion aided in excluding this diagnosis. Skanse (10) has demonstrated that under the standardized conditions employed, the 48-hour urinary excretion of tracer doses of radioactive iodine in 25 patients with thyrotoxicosis varied between 6 and 32 per cent as compared to an excretion of 53 to 84 per cent in 15 euthyroid subjects.

We have recently observed two patients, one of whom certainly, and the other most probably, developed hyperthyroidism due to clandestine ingestion of thyroid. Since the case histories are very characteristic of this category of patients it has seemed worthwhile to present them as illustrations of the use of serum protein-bound iodine determinations and especially of the urinary excretion of radioactive iodine in the differential diagnosis between alimentary and spontaneous hyperthyroidism.

CASE REPORTS

Case No. 552354. The first patient, M.McM., a 37-year-old unmarried graduate nurse, was referred to this hospital for study of recurrent symptoms of thyrotoxicosis.

Her first hospital admission (to an outside hospital) was on January 15, 1936. At that time she presented a characteristic picture of thyrotoxicosis. Her weight was 50 Kg., her pulse rate was 110 to 120 and her basal metabolic rate (BMR) was between plus 30 and plus 40 per cent. Her thyroid was bilaterally enlarged. She reported a history of urinary frequency since December 1935. She was thirsty and had urinary volumes up to 4000 cc. a day.

She was prepared for operation with iodine (Lugol's solution 10 drops three times a day). Subtotal thyroidectomy was performed on January 28, 1936, and only small strips of thyroid were left on each side. The pathological report was one of "hyperplastic goiter." Her postoperative condition was fairly satisfactory but her pulse rate remained elevated. A basal metabolic rate, taken eighteen days after operation, was plus 8 per cent. A diagnosis of diabetes insipidus was made. Pituitrin was given in an attempt to control urinary frequency but it had little effect. She was discharged on February 26, 1936.

The patient felt quite well until 1940, when she again developed almost the same symptoms as in 1936 with intolerance to heat, muscle weakness, easy fatiguability and nervousness. She was still suffering from thirst and polyuria. She was admitted to the same hospital on October 28, 1940, presenting a picture of typical thyrotoxicosis. Her weight was 41.5 Kg., pulse 100 to 110, and her BMR's were plus 32 and plus 25 per cent. Her thyroid showed a slight bilateral enlargement in the region of the upper poles.

She was again operated upon and a small mass of thyroid tissue (4 by 3 by 3 cm.) was removed from the region of the right upper pole and a similar mass (2 by 2 by 1 cm.) from the left upper pole. The pathological report was again one of "hyperplastic goiter." Her BMR thirteen days after operation was minus 14 per cent.

On January 2, 1941, the patient claimed that she developed a severe headache followed two hours later by convulsions. She was given calcium gluconate intravenously for several days, 20 units of parathormone daily for seven days and then calcium by mouth until May 5, 1941. Following this episode of convulsions her polydipsia and polyuria stopped abruptly and these symptoms have not recurred.

On July 4, 1941, she was again examined. Her BMR was minus 28 per cent and her blood calcium was 9 mg. per cent. Because of the symptoms of hypothyroidism she was given 130 mg. of desiccated thyroid daily for one month and 65 mg. daily for the two following months. This seems to have been the first period during which she took thyroid. She seemed to feel well until the beginning of 1943, when she again began to feel tired and nervous. A BMR on January 31, 1943, was plus 42 per cent. She was readmitted to the hospital on March 1, 1943, and x-ray therapy of her neck was started. She had 5 treatments during two weeks, after which she developed radiation sickness and symptoms of tetany. She was treated with large amounts of calcium, parathormone and sedatives and recovered after about ten days. Blood calcium, chlorides, phosphorus and CO_2-combining power were all within normal limits at the time of the attack of tetany. Her BMR's were falling (March 22, plus 13 per cent; March 30, plus 11 per cent; April 29, minus 4 per cent; May 1, minus 12 per cent) and her thyrotoxic symptoms abated to some degree.

She felt better for some time but in the fall of 1943 she again became very nervous and restless. She entered the hospital again November 1, 1943. At that time her BMR's were plus 46 per cent and plus 39 per cent, and her pulse rate was about 150. She was operated upon for the third time on January 4, 1944. The thyroid region was explored and a piece of tissue was removed which was thought to be thyroid, but the pathological report showed that it was thymus gland. While she was in the hospital her BMR fell to minus 6 per cent but the patient did not notice any substantial improvement. She was discharged on April 3, 1944.

In May 1944 thiourea was started, but toxic symptoms appeared ten days later, so thiouracil was substituted in a dose of 0.6 Gm. daily. After six weeks the BMR was minus 20 per cent so the dose was reduced to 0.4 Gm. daily for one month and then stopped. She felt better for a few months but later the symptoms of hyperthyroidism recurred. She was again treated with thiouracil, 0.6 Gm. daily, for about six weeks (in the spring of 1945).

Readmitted to the hospital June 16, 1945, she was still restless and nervous and had a BMR of plus 33 per cent. She was treated with Lugol's solution, high caloric diet and sedatives. This therapy appeared to have very little effect on her symptoms and on July 9 she was discharged for a prolonged rest at home on the same regimen. However, she, did not improve at all, and on September 11, 1945, she was admitted again with symptoms of irritability, restlessness and nervousness, weakness, heat intolerance, sweating and widening of palpebral fissures. Her BMR was plus 88 per cent. Several examiners found no evidence of goiter. There was no enlargement of the sella turcica, no tumor in the pelvis and no evidence of tumor of the adrenals. Thiouracil, up to 1.6 Gm. a day, did not have any effect on the symptoms of thyrotoxicosis. Her BMR on January 24, 1946, was plus 64 per cent. She was seen by a psychiatrist but there did not seem to be any psychiatric or psychological aggravating factors. The patient was discharged on March 24, 1946. She was then seen several times by her doctor. All signs and symptoms of hyperthyroidism persisted. (The history so far is based upon correspondence we had with the hospital where she was treated before the admission to the Massachusetts General Hospital.)

She was admitted to the Thyroid Clinic of the Massachusetts General Hospital on October 28, 1946, for study. At that time her history in summary revealed: A 37-year-old woman who had developed thyrotoxicosis ten years before, since which time she had had three thyroid operations (1936, 1940 and 1944), x-ray treatment (1943) and therapy with thiouracil and iodine—all without permanent relief of her symptoms of hyperthyroidism. The examination of the patient showed a thin hyperactive individual with warm, moist skin, scars in the neck after thyroid surgery, and without palpable thyroid tissue. Her eyes were slightly prominent with a stare, lid lag, and globe lag. There was a marked, coarse tremor of the hands. Some slight right facial paralysis was present but Chvostek and Trousseau signs were negative. Her weight was 44.3 Kg. and her BMR was plus 51 per cent. Her pulse was rapid and regular, varying between 110 and 150, and her blood pressure was 130/170. Urine-analysis findings were normal and routine blood counts and differential smears were within normal limits. Pelvic examination revealed a slightly enlarged right ovary, somewhat firmer than normal; her left ovary was not definitely palpable.

Thus the patient presented a clinical picture that could be explained by an overproduction of calorigenic hormone which might be either thyroid hormone or adrenalin. We thought the following possibilities had to be considered:

1. Genuine recurrent thyrotoxicosis (Graves' disease). With no thyroid tissue palpable in the neck and the right ovary somewhat enlarged, we had to consider the possibility of struma ovarii as responsible for her thyrotoxicosis.

2. Hyperadrenalinemia due to phaeochromocytoma. The history and the earlier course of her disease were definitely against such a diagnosis. The patient did not have hypertension, nor had there been observed any par-

oxysmal episodes of hypertension despite prolonged hospital observation. A phaeochromocytoma as a cause of her symptoms of many years' duration should also have produced some enlargement of the heart or other signs of heart stress. The diagnosis of phaeochromocytoma therefore was not considered to be very likely.

3. Thyrotoxicosis factitia (self-induced or alimentary thyrotoxicosis). It seemed a little peculiar to us that a patient having had three operations for removal of thyroid tissue (the third time no thyroid tissue having been found) and later x-ray treatment of the neck, should have hyperfunctioning thyroid tissue in her neck. It was, therefore, suggested that the patient was for some reason or other taking thyroid, which was responsible for her symptoms of hyperthyroidism. The following diagnostic procedure was employed:

1. Serum protein-bound iodine was determined and found to be 9.6 micrograms per cent. When compared to the normal value of 3.5 to 7, this elevated value is definitely in the range found in thyrotoxicosis.

2. A tracer dose of radioactive iodine was administered orally. The urine was collected for forty-eight hours. After that time her whole body was scanned with an external gamma-ray Geiger-Mueller counter. No radioactivity could be demonstrated in the neck, over the ovaries, or over any other part of the body.

3. The amount of radioactive iodine excreted in the urine was then determined:

0–24 hours	94.4 per cent
24–48 hours	3.8 per cent
Total in 48 hours	98.2 per cent

The almost total excretion of the radioactive iodine demonstrated that this patient did not have any functioning thyroid tissue in her body. This finding made us suspect further that the patient produced her thyrotoxic state by taking some thyroid substance. On examination of her belongings some pills were found which the patient claimed were luminal tablets given to her by her doctor. Analysis of the pills, however, demonstrated that they contained iodine. In order to test their thyroid activity they were given to an untreated patient having postoperative myxedema. Administration of 2 pills daily for a week produced a rise in BMR from minus 24 per cent to minus 13 per cent and a weight loss of 1.9 Kg. without obvious change in food or fluid intake. This test patient also experienced aching pains in her legs and hot flashes. We believed that this was proof enough to substantiate the fact that the pills were thyroid tablets.

All forms of medication were then made inaccessible to the patient. The BMR, which on admission here was plus 51 per cent, fell in less than three

weeks to normal (0 and plus 3 per cent). The protein-bound iodine fell at the same time from 9.6 micrograms per cent to 1.6 micrograms per cent, the latter value being in the range seen in hypothyroidism.

The patient was interviewed by a psychiatrist but the motivation for self-medication was not found in this single interview, nor would she admit ever having taken thyroid in excess. She was then discharged from the hospital to her local doctor.

Case No. 349932. The second patient, L. R., was a 40-year-old married woman who was referred to the Massachusetts General Hospital for study of symptoms of thyrotoxicosis.

She had been healthy until about 1935. At that time she started to reduce her weight, which was 63.5 Kg., by diet and thyroid medication. In two years she lost about 11 Kg. of weight but felt well. In the summer of 1937 she began to have series of carbuncles and boils. In October, 1938 she was hospitalized elsewhere for treatment of this condition and because of a rather sudden loss of about 5 Kg. in weight. She also complained of increased nervousness and perspiration. Her BMR was plus 49 per cent, pulse rate about 120, and blood pressure 170/90. There was no enlargement of the thyroid and no exophthalmos. The doctor who examined her felt that she presented a clinical picture of thyrotoxicosis. For a year she was treated with iodine and prolonged bed rest and received x-ray therapy to her thyroid. She continued to lose weight, decreasing to 45.5 Kg., despite a high caloric diet. Her BMR was constantly elevated, being between plus 30 and plus 60 per cent. When she did not respond to this treatment it was felt that surgical intervention was indicated and a subtotal thyroidectomy was performed in November, 1939. At operation the thyroid gland was found to be distinctly smaller than normal. A major part of it was removed. The removed gland was reported normal on microscopic examination. At operation, digital and visual search was made for aberrant thyroid gland but none was found. The patient did not improve after operation but continued to feel weak and nervous. She perspired easily and became breathless on climbing stairs. She had attacks of what her physician described as "paroxysmal fibrillations." Her weight remained at about 45 Kg.

In December, 1940 she visited another hospital where further surgery was advised but the patient refused. (The history so far is based upon correspondence with her personal physician).

She was admitted to the Massachusetts General Hospital for study on April 7, 1942. Physical examination revealed a thin, pale woman. Her weight was 38.5 Kg. Her skin was warm and moist. Brown pigmentation was present on both eyelids, and there were also dark brown, macular, circular spots (2 to 3 mm. in diameter) scattered over the cheeks and chin, the forearms and backs of the hands and around the ankles. No pigmentation was present on the palms or on the mucous membranes. There was no palpable thyroid tissue in the neck and no exophthalmos or other eye signs. A coarse tremor of the fingers was demonstrated. Her heart was of normal size, her pulse rate was 90 to 100 and her blood pressure was 130/70. An electrocardiogram was normal. BMR's were plus 31, plus 38 and plus 41 per cent. Protein-bound plasma iodine, determined by Dr. W. T. Salter, was 20.6 micrograms per cent. This value was in the range found in thyrotoxicosis, the normal being 4 to 8 micrograms per cent, according to the method used at that time. Blood cholesterol was 172 mg. per cent. X-ray examination of the chest and neck did not show any substernal goiter, or tracheal compression.

The patient presented a clinical picture of thyrotoxicosis, and the elevated serum

protein-bound iodine also favored this diagnosis, but no thyroid tissue could be felt in the neck. The possibility of a hyperfunctioning ovarian struma was considered but no evidence of ovarian tumor could be found. Therefore, the patient was discharged without a definite diagnosis on April 14, 1942.

She was admitted again to the Massachusetts General Hospital on January 1, 1947, with a diagnosis of Graves' disease. At that time her intervening history disclosed that following discharge in 1942 the patient continued very much as before. In 1944 thiouracil treatment was begun with a dose of 0.2–0.3 Gm. daily, gradually increasing to 0.6–0.7 Gm. daily after three to four weeks, and decreasing to 0.3–0.4 Gm. daily after two to three months. This course lasted for one year and was discontinued when it became apparent she received no benefit. Prior to and subsequent to this thiouracil treatment she took iodine without benefit. She continued to have exertional dyspnea and again complained of nervousness, weakness, easy fatiguability, sweating and heat intolerance. Her appetite remained good and her weight remained stationary during this period until 1946, when she gained 2 Kg.

Throughout the period following her first admission the one symptom which had progressed was the frequency of her episodes of paroxysmal rapid arrhythmia. Occurring occasionally at first, they became later more frequent, and were almost constant for the three months prior to admission. No beneficial effect was noted from quinidine or digitalis. She also took 0.6 Gm. of thiouracil a day, for the three months before admission—again without effect. Her nervousness had increased. She had not taken iodine, nor had she had a cholecystogram during the preceding year.

The examination showed a thin, pale woman weighing 47 Kg. She had cool, dry skin which was pigmented, as in 1942. There was no pigmentation in the mouth. She had a marked tremor of the fingers but no eye signs. No goiter could be felt and the presence of thyroid tissue was questionable. Her heart was slightly enlarged. Her pulse rate was 100 to 130 and her blood pressure was 140/95. An electrocardiogram showed auricular fibrillation with a ventricular rate of about 140. The blood-sedimentation rate was 5 mm. per hour. Cholesterol was 167 mg. per cent. Her BMR's were plus 36 and plus 30 per cent. The protein-bound iodine of the serum was 14.5 micrograms per cent (normal, 3.5 to 7.0 micrograms per cent).

On the whole, the patient presented the same clinical picture as she had on the first admission in 1942, the only apparent change being onset of fibrillation. She had a long history suggesting thyrotoxicosis and definite symptoms of hyperthyroidism including an elevated basal metabolic rate and definitely elevated serum protein-bound iodine. As before, the main problem was to determine whether or not this patient had endogenous thyrotoxicosis.

In her history there were several peculiar features. The patient did not improve at all following the subtotal thyroidectomy in 1939. The thyroid gland was at that time found to be grossly smaller than normal and microscopically normal. She did not respond to one year's treatment with thiouracil. She did not present a characteristic picture of thyrotoxicosis. There were no eye signs. In the period between the two Massachusetts General Hospital admissions she developed auricular fibrillation. It was of the type that appears in thyrotoxicosis. It was paroxysmal in nature, responded

poorly to quinidine and digitalis and appeared in a relatively young woman without other obvious cause. Mitral stenosis was considered but no characteristic murmurs could be heard even when the cardiac rhythm was normal. Active rheumatic fever was considered as a faint possibility, but the blood-sedimentation rate was found to be normal. A diagnosis of rheumatic fever could explain neither her high BMR nor the long clinical course.

In this situation it was decided to give the patient a tracer dose of radioactive iodine. The radioactive iodine excretion test gave the following result:

Time	*Urine volume*	*Per cent of administered I^{131} excreted in urine*
0–24 hours	680 ml.	53.2
24–48 hours	320 ml.	2.8
Total in 48 hours:		56.0

This result was in the range considered normal. Since no thyroid tissue could be felt in the neck a careful search for radioactivity over the whole body was made with an external gamma-ray Geiger-Mueller counter. This revealed no activity in the neck, over the ovaries or in any other part of the body. The results of the direct measurements therefore did not check with the urinary excretion figures. We should have been able to demonstrate some radioactivity in the body if she had excreted only 56 per cent of the dose. It was noted, however, that the urinary volumes were very small, being 680 cc. and 320 cc., and we thought that perhaps some of the urine was discarded by mistake. Both the nurses and the patient denied this possibility. We therefore decided to repeat the radioactive iodine excretion test and to collect the urine by an inlying catheter. The patient firmly objected to such a procedure but was finally persuaded to submit to it. During the test the patient asked several times to have the catheter removed because she said she had to make "important" telephone calls. However, this was not permitted. The result of the second test showed the following:

Time	*Urine Volume*	*Per cent of administered I^{131} excreted in urine*
0–24 hours	1090 ml.	85.7
24–48 hours	900 ml.	11.6
Total in 48 hours:		97.3

In this second test the urine volumes were almost double those of the first test. It is very likely that the patient herself had intentionally discarded some urine during the first test. We know by experience that the urine volume alone does not play a role in the excretion of the radioactive iodine. Since this second test revealed that the patient was excreting almost all the radioactive iodine administered, the possibility that this patient's symptoms were caused by hyperfunctioning thyroid tissue was excluded.

We were then confronted with the following contradictory evidence: 1. The patient's history and clinical picture seemed to indicate hyperthyroidism. 2. Her serum protein-bound iodine was elevated to the range found in thyrotoxicosis. 3. Her radioactive iodine excretion was almost 100 per cent. It seemed to us that the most feasible explanation of the situation was that the patient was taking thyroid. However, when this possibility was discussed with her she categorically denied it, became very upset, and immediately decided to leave the hospital. Our plans to have her seen by a psychiatrist could therefore not be carried out. The diagnosis of self-induced thyrotoxicosis is not fully proved in this patient, but there is little doubt in our minds that this must have been the essential condition.

DISCUSSION

Typically, in thyrotoxicosis one finds an elevated serum protein-bound iodine and a low urinary excretion of tracer doses of radioactive iodine. In the two cases reported here, however, an elevated serum protein-bound iodine was associated with a *high* urinary excretion of radioactive iodine, a combination which apparently is characteristic of alimentary hyperthyroidism. The serum protein-bound iodine may usually be accepted as an index of circulating thyroid hormone. It is, of course, impossible to differentiate between the thyroid hormone produced by the individual's own gland and thyroid hormone derived from medication. However, the radioactive iodine tolerance test is of assistance in determining the origin of a high value of "hormonal" iodine. If the elevated protein-bound iodine is caused by an increase in activity of the patient's thyroid gland then this gland's avidity for iodine is also increased and this can be demonstrated either directly by *in vivo* measurements of radioactivity over the gland or indirectly by a low urinary excretion of the radioactive iodine. If, on the other hand, the high serum protein-bound iodine is caused by prolonged administration of thyroid, then the gland has a decreased avidity for iodine and the radioactive iodine test gives a high excretion value.

It might be argued that the two patients whose cases are reported here probably had little or no thyroid tissue left and therefore would be expected to excrete practically all of the administered radioactive iodine. However, even in a patient with an intact thyroid gland, the same pattern

of elevated serum protein-bound iodine and high excretion of radioactive iodine may be produced by excessive ingestion of thyroid, as illustrated by the following case:

A.P. was a 35-year-old married woman who was healthy until she reached puberty at the age of 14. Shortly after this she began to have attacks of somnolence, and a diagnosis of narcolepsy was made by an outside physician. At that time she was found to have a low basal metabolic rate of from minus 25 to minus 32 per cent. For the past five years her physician had prescribed fairly large doses of thyroid; for the first three years she took 0.3 Gm. daily and for the last two years, 0.4 to 0.5 Gm. daily. She developed palpitation and dyspnea on exertion, but denied nervousness or intolerance to heat. Examination revealed a jumpy and nervous woman. Her skin was of normal temperature but somewhat moist and there was a fine tremor of the extended fingers. Her thyroid was palpable but not enlarged. No eye signs were present. Her heart was of normal size. Her pulse rate was 86. Her blood pressure was 130/70. The basal metabolic rate was plus 3 per cent. Her serum protein-bound iodine was 9.4 micrograms per cent and she excreted 86.7 per cent of a tracer dose of radioactive iodine in forty-eight hours.

After these studies were completed the thyroid medication was stopped. Eight weeks later the patient had gained 6 Kg. in weight. She was much more quiet than she had been at the first examination. The tremor of her fingers had disappeared. Her pulse rate had fallen to 68. At this time the laboratory studies were repeated. Her basal metabolic rate was minus 17 per cent. Her serum protein-bound iodine was 5.4 micrograms per cent and the radioactive iodine excretion test showed 66.8 per cent excreted in forty-eight hours.

When this case was first studied we found the combination of elevated serum protein-bound iodine and high excretion of radioactive iodine which we had found in the two other cases. When the thyroid medication was stopped, both the serum protein-bound iodine and the excretion of radioactive iodine decreased to values definitely in the normal range. The result of the radioactive iodine excretion test suggests that the thyroid medication depressed the function of the thyroid gland, as would be expected from previous observations (Farquharson and Squires (11); Riggs, Man and Winkler (6)).

Unfortunately, the combination of an elevated serum protein-bound iodine and high urinary excretion of radioactive iodine, while characteristic of, is not by itself pathognomonic of, alimentary thyrotoxicosis. In a patient with endogenous thyrotoxicosis treated with either thiouracil or iodide one may find the same laboratory pattern until the disease is brought under control. The radioactive iodine excretion, which in the untreated thyrotoxic patient is low, becomes high when thiouracil treatment is used because this drug prevents the utilization of iodide by the thyroid gland. Therefore, when a tracer dose is administered, most of it will be excreted in the urine. When iodide is given therapeutically, the concentration of inorganic iodide in the blood is so great compared to the amount of iodide administered in a tracer dose, that differences in the thyroid's avidity for

iodide will not be demonstrated. Furthermore, the gland becomes saturated with iodide and will collect very little more.

A patient who has recently had a cholecystogram may also show the same pattern of laboratory data. The organically bound iodine in the gallbladder dye may keep the serum protein-bound iodine elevated for a long period of time, perhaps as long as a year (12). It will also increase the excretion of radioactive iodine. As an example, we recently studied a patient with a classical picture of Graves' disease. Untreated, the patient excreted only 12.6 per cent of a tracer dose of radioactive iodine. She then had a cholecystogram. Two days later the radioactive iodine test was repeated. In the second test the patient excreted 79.5 per cent. We do not know how long this effect on the radioactive iodine excretion lasts nor the mechanism by which it works. Experiments to elucidate these problems are in progress in our laboratory. We believe, however, that such things as previous cholecystograms might be responsible for the fact that thyrotoxic patients occasionally have a very high excretion of radioactive iodine.

SUMMARY

A case of secretly self-induced thyrotoxicosis (thyrotoxicosis factitia) is described, and a second case in which circumstantial evidence strongly favored the same diagnosis is also discussed. In both patients the clinical picture was indistinguishable from that of endogenous thyrotoxicosis. The differential diagnosis was facilitated by the use of tracer doses of radioactive iodine. In endogenous thyrotoxicosis the urinary excretion of radioactive iodine is low compared to that of euthyroid individuals. In thyrotoxicosis factitia the excretion of radioactive iodine is higher than normal. Since the serum protein-bound iodine is elevated independently of the source of iodine-containing hormone, this test is not contributory to the differential diagnosis. Other conditions which may produce the same combination of elevated serum protein-bound iodine and high excretion of radioactive iodine are discussed.

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HORMONAL FACTORS INVOLVED IN THE REGULATION OF BASAL BODY TEMPERATURE DURING THE MENSTRUAL CYCLE AND PREGNANCY*

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THE biphasic aspect of the basal body temperature during the normal menstrual cycle is now a well-established fact. The temperature rise of three-fifths to one degree Fahrenheit which occurs at or about the time of ovulation is a phenomenon frequently used diagnostically and therapeutically. There are frequent variations from this typical biphasic curve, with its abrupt midcycle rise, which have been commented upon elsewhere (1), but generally speaking the rise maintains itself until the onset of the next menstrual period during which it drops to its preovulatory level. In the event that pregnancy occurs and the next menstrual period fails to appear when expected, the basal temperature does not return to its preovulatory level, but continues at or about the level maintained during the latter two weeks of the menstrual cycle. This, in itself, is a fairly accurate diagnostic sign of pregnancy.

The purpose of this paper is two-fold: first, to present evidence as to the actual cause of the temperature rise during the latter half of the menstrual cycle and second, to investigate the progress of the basal body temperature throughout pregnancy.

METHODS AND OBSERVATIONS

In order to ascertain the effect of steroid ovarian hormones on basal body temperature, six patients with secondary amenorrhea and with little or no endometrial activity as determined by biopsy, were studied in the following manner. After a temperature base line had been established, the patients were given by mouth a priming dose of estrogen consisting of 3 to 5 mg. of either estrone sulfate[1] or diethylstilbestrol daily for about two weeks. Various amounts of progesterone, ranging from 10 to 25 mg. daily,

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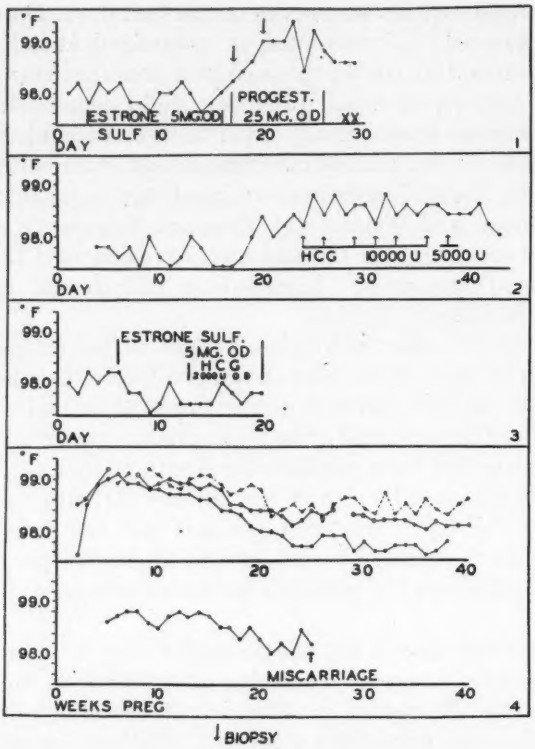
[1] The estrone sulfate (Premarin) and chorionic gonadotropin (APL) were supplied to us through the courtesy of Dr. Murray Scott of Ayerst, McKenna and Harrison; the progesterone (Proluton), through the courtesy of Dr. Edward Henderson of the Schering Corporation.

were then administered for from seven to fourteen days. During this time the estrogen was either discontinued or maintained at half the original dosage. The temperature curves obtained from these patients during treatment indicate that estrogen may produce a slight depression of the basal temperature whereas progesterone, either with or without estrogen, produces a very definite rise. In some instances the latter amounted to a degree or more (Fig. 1). These observations are in substantial agreement with the findings of Barton and Weisner (2). Frequent biopsies to ascertain the effect of these substances on the endometrium confirmed the activity of the administered hormones. Characteristic endometrial responses are demonstrated in Figures 5 and 6.

In addition to this effect of ovarian steroids on the temperature levels of amenorrheic patients, it has been shown previously that the administration of large amounts of chorionic gonadotropin to normal women during the latter half of the menstrual cycle maintains the postovulatory rise and prevents menstruation for a considerable length of time (3). This experiment has been repeated by one of the authors (4) with confirmation of these findings (Fig. 2). It has been assumed that the chorionic gonadotropin stimulates the existing corpus luteum to produce progesterone and that the latter substance is responsible for the maintenance of the temperature rise.

In this connection there is also the possibility that the chorionic gonadotropin itself is directly responsible for the maintenance of the temperature rise. To test this alternative, a 31-year-old bilaterally ovariectomized woman was given an equivalent amount of chorionic gonadotropin after priming with estrogen. No rise in temperature occurred (Fig. 3). It therefore seems probable that the maintenance of the temperature rise during treatment in the normal woman is due to the action of progesterone secreted by the ovary in response to the injected gonadotropin.

In view of these findings one would expect that the elevated basal body temperature would be maintained throughout pregnancy, since progesterone is present in gradually increasing quantities, at least as determined by measurement of the pregnandiol excretion (5). To test this hypothesis, three patients were instructed to continue temperature charts throughout the period of gestation. One woman kept records during two pregnancies, the second of which terminated in a five and one-half months miscarriage due to unknown causes. It is interesting and surprising to note that in each case the temperature in the fourth month of pregnancy began a gradual drop which reached preovulatory levels by the beginning of the fifth month (Fig. 4). Since these observations were made, two similar charts have been included in a report concerning the time of ovulation and conception (6).



FIGS. 1–4

1. Temperature chart of patient treated with estrone sulfate, 5 mg. daily, followed by progesterone, 25 mg. daily. Arrows indicate dates of endometrial biopsy. ×× indicates onset of bleeding.

2. Temperature chart of a normally-ovulating patient treated with human chorionic gonadotropin following the ovulatory temperature rise.

3. Temperature chart of a bilaterally ovariectomized woman treated with daily doses of human chorionic gonadotropin following previous treatment with estrone sulfate.

4. Temperature charts of 3 patients followed throughout pregnancy, the base line representing the number of weeks since the last menstrual period. One patient recorded her temperature throughout a second pregnancy, which terminated in a miscarriage at 25 weeks.

DISCUSSION

It is apparent from the experimental data presented above that the elevation of basal body temperature in hormone-treated amenorrheic women is due to the action of progesterone. That this is also the case in normal women during the latter half of the menstrual cycle and early

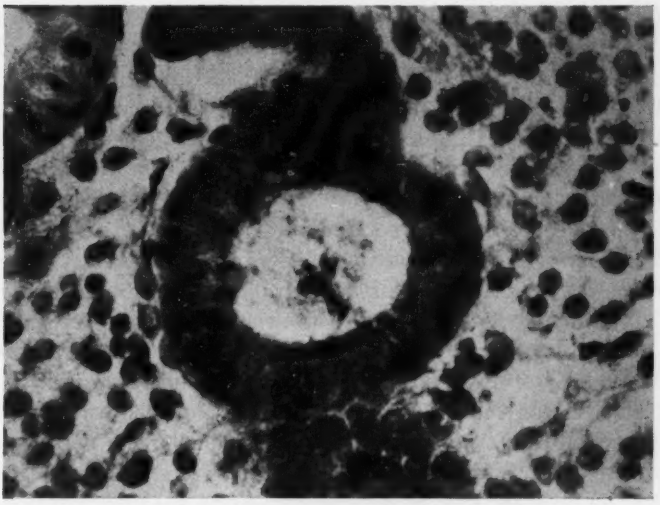


FIG. 5. Photomicrograph of an endometrial gland from the first biopsy indicated in Figure 1, to demonstrate the response to the estrogen treatment.

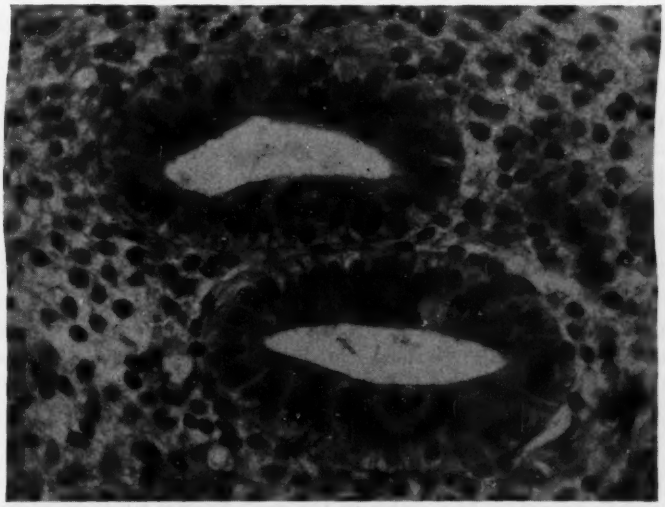


FIG. 6. Photomicrograph of endometrial glands from the second biopsy indicated in Figure 1, to demonstrate the response to the progesterone treatment.

pregnancy is strongly suggested by the close temporal correlation between the rise and decline of basal temperature and luteal activity. On the other hand, if this is the case, it is surprising that a temperature drop should occur during midpregnancy at a time when pregnandiol excretion studies indicate a constantly rising level of circulating progesterone.

Several possibilities exist which might account for the above discrepancy. For instance, it is possible that a refractory state develops in connection with the thermogenic action of progesterone or that the action of progesterone is overcome by stronger thermodepressant factors. Lack of data in this regard precludes further speculation. A second alternative is that the maintenance of the temperature rise during early pregnancy may be due, at least in part, to unknown factors other than progesterone. Here again the paucity of information makes the identification of any such factors a matter of conjecture. It is interesting to note, however, that Caffier (7) has reported that trophoblast grown in tissue culture possesses proteolytic activity which disappears about the fourth month of gestation. Could it be that the lytic activity of the trophoblast on the decidual tissue of the mother during the early months of pregnancy results in a slight febrile response on the part of the latter? Certainly the time relationship is interesting and since widespread lysis of tissue often produces a febrile response, this possibility may have some factual significance.

An additional explanation with important implications is that the decline in basal body temperature during pregnancy, as in the menstrual cycle, may reflect a decrease in physiologically active progesterone. This concept is at variance with the generally accepted belief that the maintenance of gestation is dependent upon increasingly high levels of progesterone activity. However, Atkinson and Hooker (8) have recently pointed out that the prevalent view is open to question. Urinary excretion of pregnandiol in woman is accepted as evidence of the presence of circulating progesterone. Unfortunately, however, few or no direct data are available relating the levels of circulating progesterone to the urinary output. Furthermore, experimental studies in lower animals indicate that the level of physiologically active progesterone may decrease considerably during the second half of gestation (8, 9, 10). The decline of the elevated basal temperature at midpregnancy in the human is consistent with a hypothetical decline of active circulating progesterone. Further work is indicated before the question can be settled conclusively.

SUMMARY AND CONCLUSIONS

In order to ascertain the cause of the well known postovulatory rise in basal body temperature in normal cyclic women, six patients with amenorrhea and with little or no endometrial activity were given sequential ther-

apy of estrogen followed by progesterone. The patients were followed by temperature charts and endometrial biopsy. The basal body temperature invariably rose significantly (three-fifths to one degree Fahrenheit) during progesterone therapy.

The postovulatory temperature rise was also maintained and menstruation postponed ten to thirty days in normal women by the administration of chorionic gonadotropin. However, chorionic gonadotropin administered to a castrate following estrogen priming did not result in any significant change in basal temperature. It was assumed, therefore, that the maintenance of the postovulatory temperature rise by chorionic gonadotropin was due to its luteotropic effect and that here also progesterone produced the temperature response.

To determine whether the rise were maintained throughout gestation, three women (one through two pregnancies) kept temperature charts until delivery. The temperature fell to its preovulatory level uniformly between the fourth and fifth month in all cases. This was in spite of the commonly supposed steady rise in progesterone activity as determined by pregnandiol excretion. Some conjecture, but no final explanation is offered for this interesting phenomenon.

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THE CAUSE OF PHYSIOLOGIC BASAL TEMPERATURE CHANGES IN WOMEN*

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THE clinical application of basal temperature changes in the study of ovarian function has met with wide acceptance since the publications of Tompkins (1), Davis (2) and others (3, 4, 5). This simple physiologic phenomenon has provided a much-needed tool for the interpretation of normal and abnormal ovarian activity. The mechanism for these temperature fluctuations has not been elucidated. The following observations were carried out in an attempt to throw additional light on this subject.

In the past three years well over 1000 temperature graphs have accumulated in the study of sterility and other endocrine disorders, as well as in a large group of normal individuals who were used as controls. These graphs have been prepared by the patients themselves after careful instructions on how to keep a basal temperature record. Oral temperatures have been used throughout, since it has been demonstrated that the pattern is similar to rectal temperatures and that the latter provide no greater degree of accuracy. Most patients have obtained readings over a period of three or four months but many have kept a continuous graph for a year or longer.

A careful study of these graphs reveals some interesting facts which are worthy of comment. About 75 per cent of patients present graphs in which the temperature curve is sufficiently typical to provide valid data concerning ovarian function. The remaining 25 per cent of patients have such violent temperature fluctuations that the pattern may be completely disturbed. These are highly strung, nervous individuals, many of whom lead irregular lives, working, eating, sleeping and worrying to such an extent that these emotional, physical and metabolic stimuli mask the hormonal control of the body basal temperatures. Furthermore, in the interpretation of temperature graphs isolated irregularities in a curve must not be given undue importance because the general pattern extending over one or more complete cycles is a better index of ovarian activity. In spite of these handicaps, a temperature graph carefully prepared by a patient is of inestimable value.

There has been considerable discussion as to the interpretation of the

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ovulatory rise in temperature in the light of its great practical significance. Clinically, as well as for academic reasons, it is important to know the exact time of ovulation. The life of the human ovum is very short, certainly less than twenty-four hours. Spermatozoa survive a longer period but their fertilizing power is definitely limited. Problems of human reproduction would be simplified if we could indicate the exact time of ovulation.

In some graphs a temperature drop can be seen just before the ovulatory rise, usually the day before. Some authors have interpreted this preovulatory drop as the time of ovulation. Coitus during this period has resulted in some pregnancies. However, the drop is not a constant phenomenon and is difficult to interpret. In the graphs we studied, a typical drop prior to the ovulatory rise was evident in less than fifty per cent. Furthermore, this drop in temperature could be ascertained in many cases only after the ovulatory rise occurred. Thus, its clinical value is lost because of the inability of the patient to interpret the graph correctly.

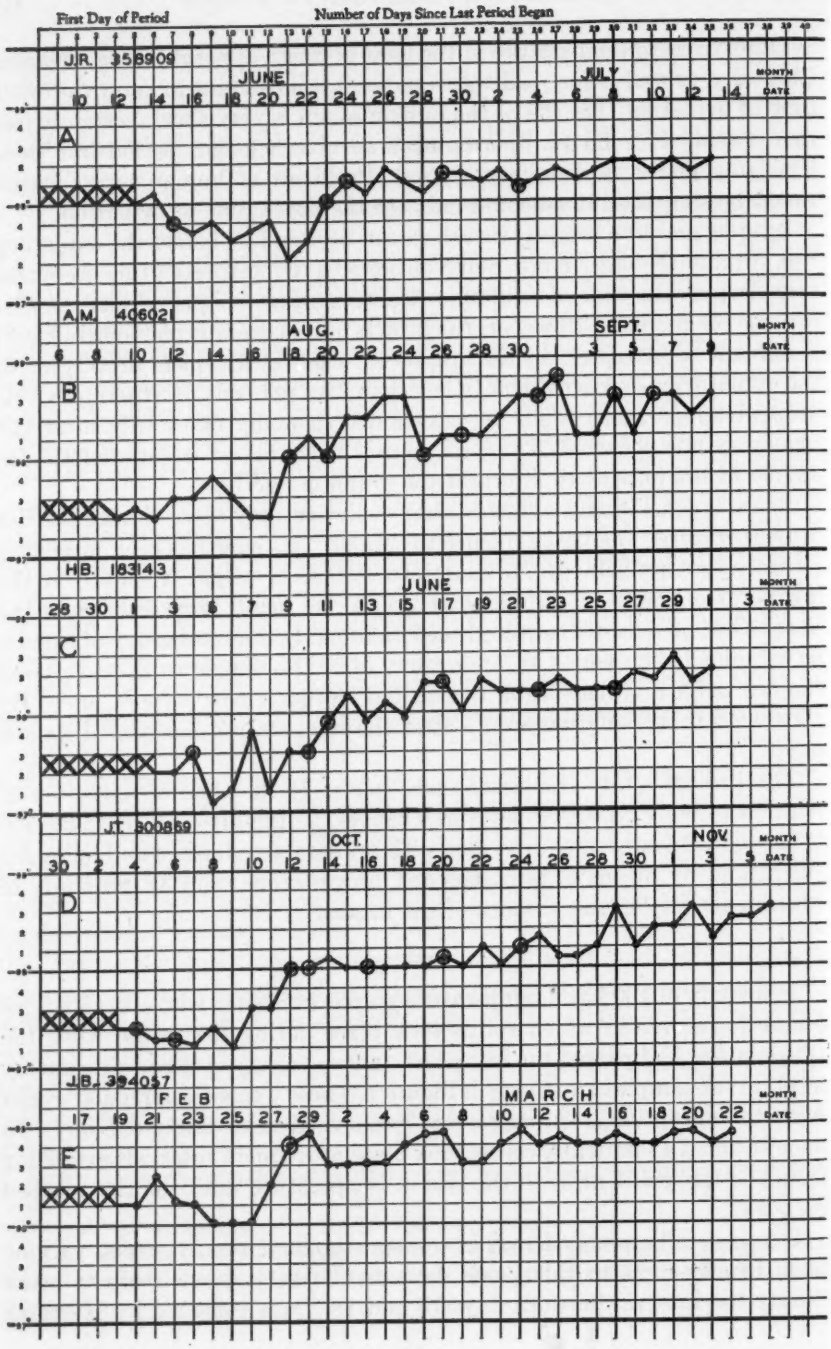
There is considerable evidence that ovulation may occur during the rise in temperature. The best evidence to support this view has been presented recently by Greulich (6). He examined ovaries of women removed at the low point in the basal temperature graph. He found large follicles in the ovaries but no recently ruptured corpora lutea. In a second group of women he examined the ovaries at laparotomy after the temperature had passed the low point and had begun to rise and he found new corpora lutea. He concluded that ovulation occurs after the temperature has passed its low point.

The present study comprises three parts: 1) the time of ovulation in relation to body basal temperature changes; 2) the artificial reproduction of the temperature curve in women with primary amenorrhea and following castration, by means of estrogens and progesterone; and 3) the study of ovarian activity in the absence of the uterus.

DATA

In a study of 100 basal temperature graphs of women who conceived, we found 24 records in which coitus took place during the period when the temperature had passed the low point and was rising to the postovulatory level. Five examples are shown in Figure 1. These women had regular cycles prior to conception, recurring every twenty-seven to thirty-one days. In an additional 48 patients, coitus took place at frequent intervals extending from the low point prior to the rise in temperature until the rise reached its peak.

It is generally assumed that in women who have regular cycles varying from twenty-eight to thirty-one days, ovulation is most likely to occur during the interval between days 10 and 18. Data collected by Hartman


First Day of Period
Number of Days Since Last Period Began
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(7) in the macacque, as well as records of isolated coitus in women confirm this observation. Furthermore, the life of the human corpus luteum has been estimated to be fourteen or fifteen days. In Figure 1A, coitus occurred on days 7, 15, 16 and 21. It is unlikely that the fruitful coitus took place on day 7 or 21. Thus there is good presumptive evidence that ovulation and pregnancy occurred on day 15 or 16 during the rise in the temperature curve, rather than on day 13, the low point in the curve preceding the rise. In Figure 1B, coitus occurred on days 13 and 15 and not again until day 20. It is fair to presume that fertilization occurred during one of these days and during the temperature rise. Similar deductions have been drawn from data in Figures 1C and 1D. Finally, Figure 1E shows a graph from a patient who had regular menstrual cycles recurring every twenty-eight to thirty-one days. She had not conceived because of a severe oligospermia in the husband. For this reason the frequency of coitus was reduced to once or twice a month. Thus coitus took place only once, on day 13, and it was followed by conception. This coincided with the peak of the luteal rise in basal temperatures.

The present study was not contemplated at the time the patients prepared their graphs. The women were primarily patients who complained of infertility and a basal temperature record was a part of the investigation and treatment of their failure to conceive.

There is considerable evidence that the high level of the temperature during the luteal phase of the cycle is brought about by progesterone. The

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FIG. 1. Basal temperature graphs in a study of sterility in patients who ultimately conceived, showing the fertile cycle in each case.

(A) J. R. 358909. This patient had fairly regular 29 to 31-day cycles as indicated in her temperature graph. Coitus, marked by a circle, occurred on days 7, 15, 16 and 21. She probably conceived on day 15 or 16 as the temperature was reaching the top of the rise.

(B) A. M. 406201. This patient had regular 27 to 28-day cycles. Coitus occurred on days 13 and 15 and not again until day 20. She most likely conceived on day 13 or 15 after the temperature had passed its low point on day 12.

(C) H. B. 183143. The ovarian cycles were fairly regular, varying from 28 to 31 days. The low point in the temperature curve occurred on day 12: however, coitus took place on days 8, 14, 15 and 21. She probably conceived on day 14 or 15.

(D) J. T. 300869. This patient had regular 27 to 29-day cycles. The low point in the temperature curve was on day 10. Coitus occurred on days 5, 7, 13, 14, 17 and 21. It is more than likely that pregnancy occurred on days 13 or 14.

(E) J. B. 394057. This patient had regular cycles recurring every 28 to 31 days. Her husband had a pronounced oligospermia. Coitus occurred only once, on day 13 in the cycle illustrated, following which she conceived. Note that this was near the peak of the ovulatory rise.

following facts support this view. The temperature remains at its elevated level during the active secretory life of the corpus luteum. We have been able to correlate the output of pregnandiol and the elevated temperature. The rise in temperature is followed by an increased output of pregnandiol which persists throughout the luteal phase. Twenty-four to thirty-six hours prior to menstruation the corpus luteum ceases to be active, pregnandiol

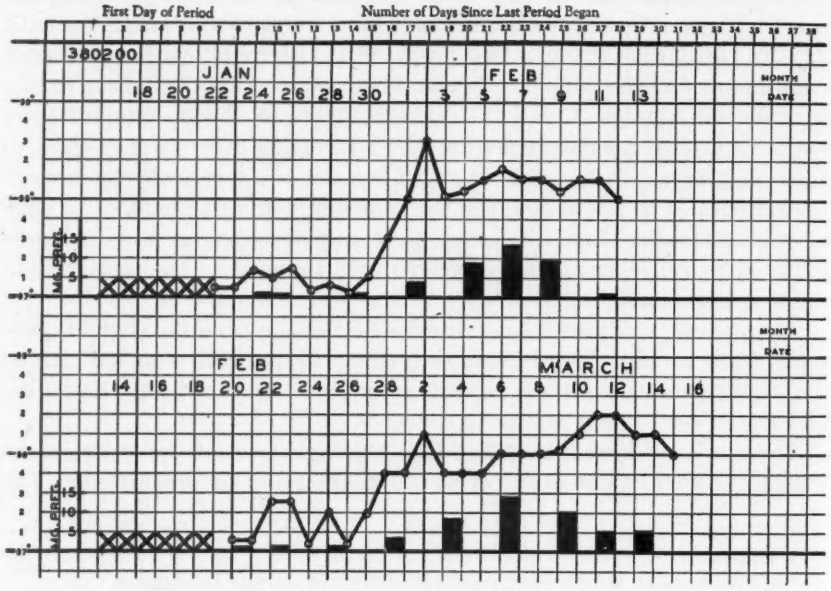

FIG. 2. Two menstrual cycles, 28 and 31 days in length, showing the relationship of pregnandiol excretion in the urine (black columns) and the basal temperature fluctuations. There is a good correlation between the excretion of pregnandiol and the luteal rise in the temperature.

levels drop rapidly and the temperature curve slides down to the preovulatory level.

In a small group of young women with regular menstrual cycles who were studied in the clinic, an attempt was made to correlate the temperature graph and the output of pregnandiol in the urine. In Figure 2 it can be seen that very small amounts of pregnandiol, probably extra-ovarian in origin, are recovered prior to the temperature rise. The first appreciable amount of pregnandiol appears after the rise has begun. The daily output increases, reaching its maximum about the middle of the luteal phase. There is no way of correlating ovulation, corpus luteum activity and pregnandiol output more accurately, for there must be a lag between proges-

terone excretion by the corpus luteum and its metabolism and conversion into pregnandiol.

If pregnancy occurs, the temperature fails to drop and continues at the postovulatory level throughout the continued active life of the corpus luteum. Furthermore, the postovulatory rise in temperature persists for approximately fourteen or fifteen days, regardless of the length of the cycle, since this is the approximate life of the corpus luteum as a secretory gland. Lastly, during an anovulatory cycle there is no rise in temperature, and it remains at approximately the same level until bleeding ensues.

The second part of this presentation concerns itself with the artificial production of typical body basal temperature fluctuations by the administration of estrogens and progesterone,[1] in women with no ovarian activity. Two groups of young women were selected: 1) four young women with primary amenorrhea who had been studied in our clinic for many months; and 2) four young women in whom it had been necessary to remove the ovaries, tubes and uterus because of disease of these pelvic organs. Basal temperature readings were charted over a four-month period, and their curves were typical, in that none exhibited any sustained rise and readings fluctuated from day to day as in the male. Pregnandiol determinations were made from 24-hour urine collections at periodic intervals, but little or no pregnandiol was present. It was therefore assumed that these young women had no ovarian activity of their own.

Ethinyl estradiol was administered orally to two patients in each group. They received 0.3 milligram daily throughout this first part of the experiment. About two weeks after the onset of therapy, 10 milligrams of progesterone in oil was given intramuscularly daily for twelve to fifteen days, following which progesterone and estrogen were discontinued. The temperature graphs were similar in the four patients. Examples are shown in Figures 3 and 4.

The estrogenic substance produced no change in the characteristics of the curve prior to medication. Within twenty-four hours after the patient received progesterone the temperature rose abruptly, perhaps more so than in the normal cycle. It remained elevated during the entire period in which the patient received progesterone. However, following the discontinuation of estrogens and progesterone the temperature dropped slowly to the general level existing during the estrogenic phase. The decline was very slow, lasting three to four days, which is much longer than in the natural cycle. In general, the curve of the recovery of pregnandiol from the urine

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[1] We wish to thank Dr. Edward Henderson and the Schering Corporation for liberal supplies of progesterone and estrogens used in this study.

in all of these patients followed the temperature curve, but the amount of pregnandiol recovered varied in each patient.

It can be said that the basal temperature curve of the artificial hormonal

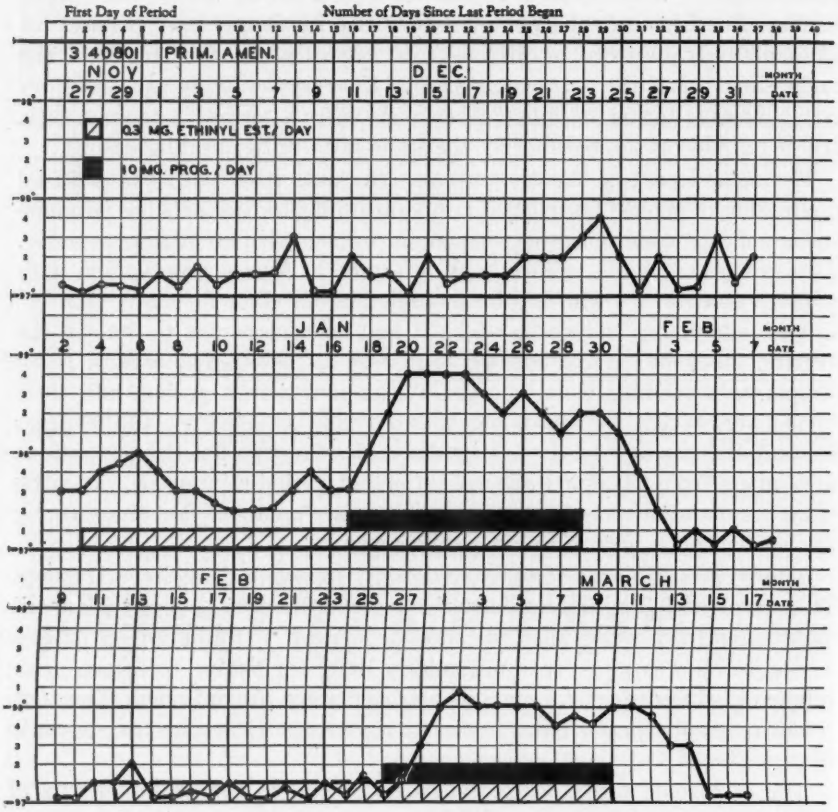

FIG. 3. The basal temperature graph of a patient with primary ovarian failure. Note the irregularity of the curve prior to the oral administration of ethinyl estradiol. During the estrogenic phase the temperature remains low and fairly constant. Following the parenteral administration of progesterone the rise is rather abrupt but after the cessation of medication the decline is more gradual. The artificial hormonal cycle thus induced simulates the natural ovarian cycle. The length of the estrogenic and progesterone phases can be varied at will.

cycle induced in these patients with no ovarian function of their own resembled the natural cycle with two exceptions. The rise was more rapid and the decline was more prolonged in the artificial cycles than in most natural ones. The rapid rise may be due to the sudden intake of a consider-

able amount of progesterone whereas in the normal cycle the developing corpus luteum may liberate progesterone more slowly. It may likewise vary with the rate and amount of secretory activity in different individuals thereby altering the pattern of the luteal phase of the cycle.

As a control for these observations one cubic centimeter of cottonseed oil was substituted for the 10 milligrams of progesterone in one cubic centimeter of oil. The rest of the experiment was not varied. There was no rise in basal temperature, and the pattern of the curve did not change. This

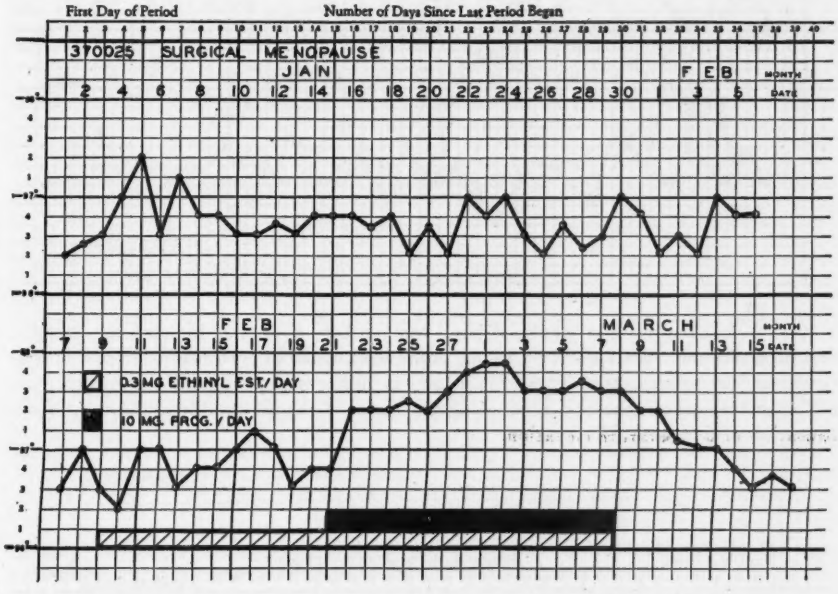

FIG. 4. The basal temperature graph of a 28-year-old patient who had undergone a surgical menopause. Note the low level of the curve prior to medication. The administration of estrogen did not alter this curve. However, progesterone produced a prompt rise and this elevated temperature continued until the cessation of therapy.

removed the parenteral mode of administration of the progesterone as a factor in the elevation of the temperature above the estrogenic level (Fig. 5).

In three of the patients, pregneninolene was substituted for progesterone in oil; otherwise the experiment was not altered. Fifty milligrams of Pranone was administered by mouth daily. The only change in the pattern of the artificial cycle was the slow rise of the basal temperature after the administration of pregneninolene orally, in comparison to the rapid rise following progesterone in oil (Fig. 6).

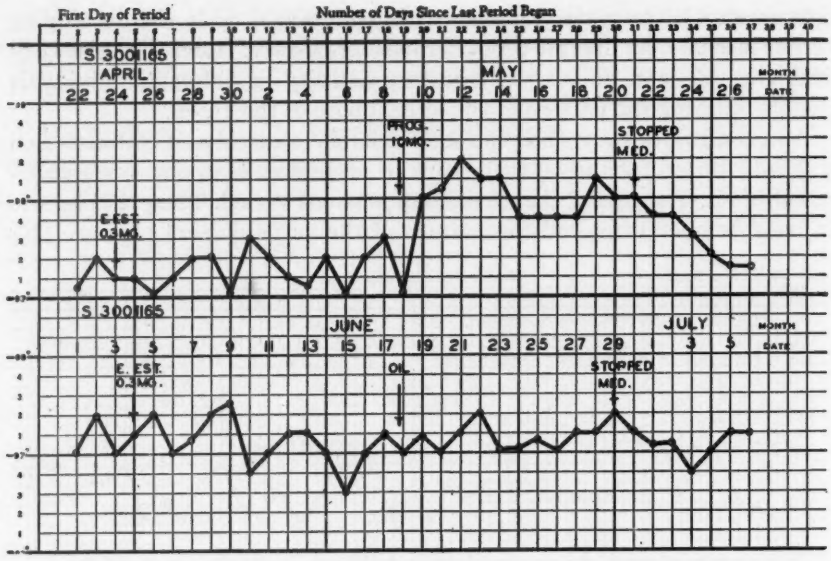

FIG. 5. In this graph an artificial basal temperature cycle was induced by estrogen and progesterone. In a second cycle one cubic centimeter of cottonseed oil was substituted for the progesterone. Note that no rise in temperature followed the substitution of the cottonseed oil, thereby eliminating the factor of parenteral administration in the causation of temperature elevation.

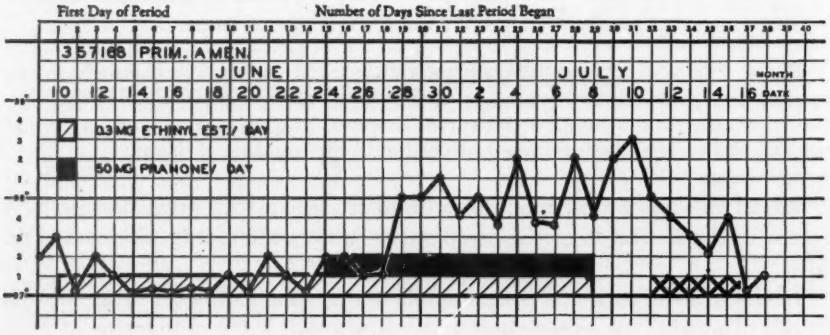

FIG. 6. In this artificial basal temperature cycle pregneninolene, an active oral progestin, was substituted for progesterone in oil. Note the gradual rise in the temperature in contrast to the more abrupt rise following the administration of progesterone parenterally. This patient exhibited typical progesterone withdrawal bleeding.

In a previous observation methyltestosterone was administered to young women with regular cycles who complained of severe dysmenorrhea. Basal temperature graphs have been kept by these women during the last

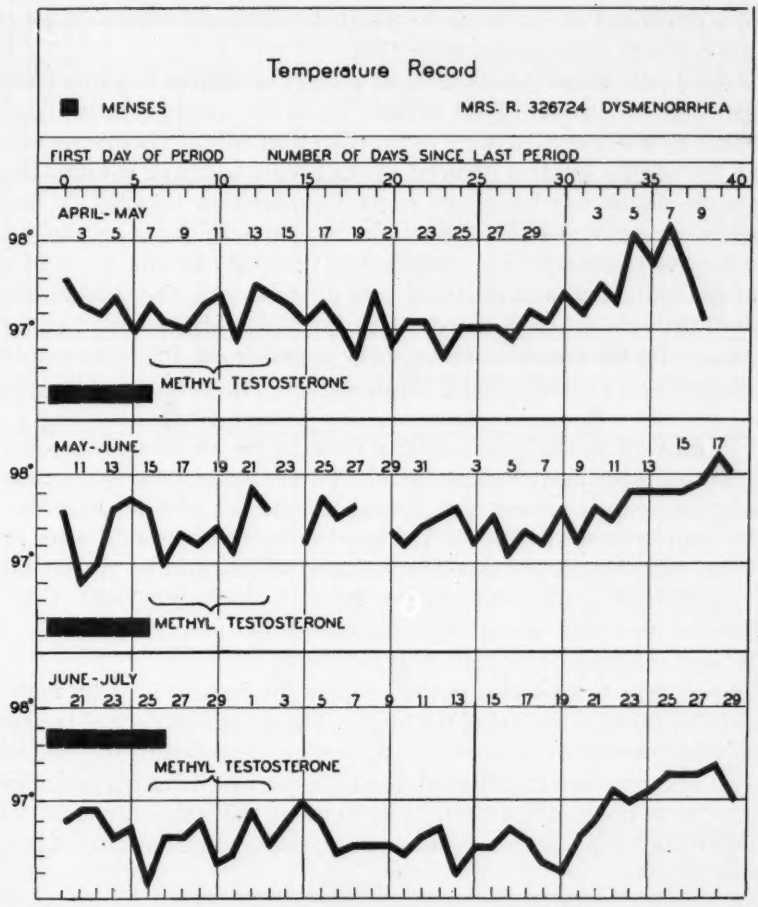
Temperature Record
MENSES
MRS. R. 326724 DYSMENORRHEA
FIRST DAY OF PERIOD
NUMBER OF DAYS SINCE LAST PERIOD
0 5 10 15 20 25 30 35 40
APRIL-MAY
3 5 7 9 11 13 15 17 19 21 23 25 27 29 3 5 7 9
98°
97°
METHYL TESTOSTERONE
MAY-JUNE
11 13 15 17 19 21 23 25 27 29 31 3 5 7 9 11 13 15 17
98°
97°
METHYL TESTOSTERONE
JUNE-JULY
21 23 25 27 29 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
98°
METHYL TESTOSTERONE
97°

FIG. 7. The administration of methyltestosterone for the alleviation of dysmenorrhea does not produce an elevation of the temperature during the estrogenic phase. It often results in a prolongation of the cycle and suppression of ovulation.

three years. Our routine is to prescribe 30 milligrams of methyltestosterone orally each day beginning on day 7 of the cycle and continuing for eight days, until a total of 240 milligrams has been given during the cycle. The subsequent menstrual period is delayed considerably but it is likely to be

painless or associated with only moderate discomfort. Many of the cycles are anovulatory in character. The pattern of the basal temperature curve resembles that of the estrogenic phase. The general level may be somewhat lower than in the natural cycle. A rise in the temperature may occur at the end of a prolonged cycle. It can be concluded that testosterone does not produce a rise in body temperature (Fig. 7).

The third part of this presentation is a study of ovarian function following the surgical removal of the uterus. There are ample clinical data to show that ovaries may continue to function normally for many years following the careful surgical removal of the uterus in young women. However, some doubt has been cast on this supposition because, in many instances, symptoms of the climacteric develop within one or two years following hysterectomy. It is possible that this may be the result of the age of the individual and a lack of care in conserving the ovarian blood supply. Furthermore, there is some experimental evidence that the uterus is necessary for the normal metabolism of progesterone. Basal temperature studies provided a useful tool for obtaining new data concerning this problem.

In an attempt to study the ovarian cycle in the absence of the uterus, the following experiment was carried out. Twelve women who had complete hysterectomies for a variety of pathologic conditions, with the retention of one or both normal ovaries, started basal temperature graphs after they recovered following their surgical procedures. All had normal ovarian function and typical temperature curves prior to their operations. Urinary pregnandiol was determined for a 24-hour period once a week. The patients were followed for at least four months.

Figure 8 depicts a portion of the temperature records kept by four patients following the removal of the uterus. The general pattern of the curve is typical of normal ovarian activity recurring at approximately the same interval as the patient experienced when she was menstruating periodically prior to the removal of the uterus. The length of the temperature elevation was approximately fourteen or fifteen days, the average duration of corpus luteum activity.

Progesterone metabolism, as measured by the urinary output of pregnandiol during a 24-hour period was not altered. Minute quantities were present during the low level of the temperature prior to ovulation. These small amounts are found in normal women during the estrogenic phase of the cycle and may represent adrenal activity. Considerable amounts, ranging from 5 to 17 milligrams in a 24-hour period, were recovered during the phases of elevated temperature.

We concluded that follicles grow to maturity, rupture, and become functional corpora lutea which have a normal life cycle even in the absence of

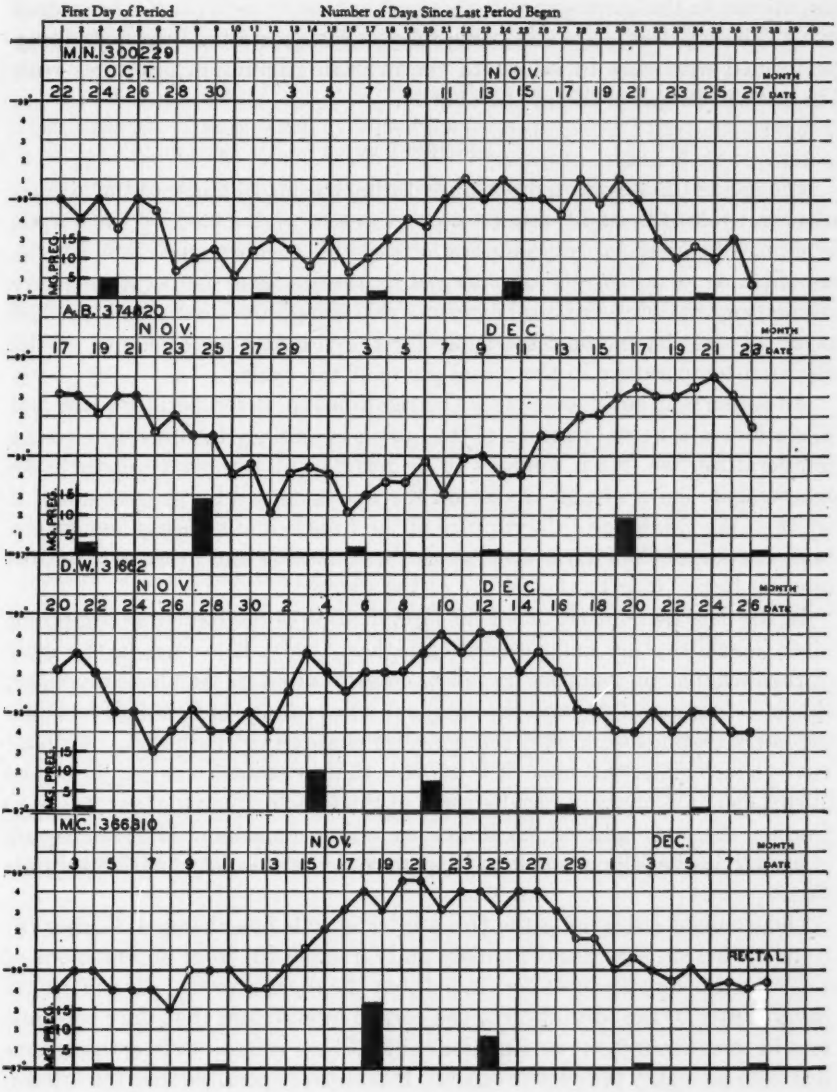

FIG. 8. Natural ovarian cycle in 4 young women each of whom had a complete hysterectomy. The temperature graph indicates a normal estrogenic phase and a normal luteal phase. Pregnandiol in the urine (black columns) was absent during the estrogenic phase and present in normal amounts during the luteal phase indicated by the elevated temperature.

the uterus. In the human the uterus is not necessary for a normal hormonal cycle. These patients are still being followed to determine if hysterectomy performed with care to safeguard the ovarian circulation interferes with the natural length of life of the ovaries.

DISCUSSION

The rise in basal body temperature during the luteal phase of the normal ovarian cycle is brought about by progesterone elaborated by the corpus luteum. The artificial reproduction of the normal hormonal cycle by the substitution of estrogens and progestins results in a temperature curve simulating that of the natural ovarian cycle. Progesterone can be identified positively as the factor responsible for the rise and maintenance of the elevated temperature in the luteal phase of the cycle.

An important consideration is the relationship of temperature rise and ovulation. The actual rupture of the follicle is unlikely to contribute to the temperature rise, for it appears to be insufficient trauma to disturb the temperature curve. In all likelihood the rise is caused by progesterone. If so, then the rise follows the rupture of the follicle and is dependent on the luteinization of the follicle wall, unless luteinization and progesterone production begin prior to the release of the ovum.

In the past we have regarded the luteinization of the follicle as beginning after follicle rupture and proceeding rather slowly. However, in the light of the fact that pregnancies do follow coitus limited to the phase involving the rise of temperature, and the excellent observations of Greulich correlating early corpora lutea and temperature changes in the human, we may have to reconsider this idea. It is possible that luteinization actually begins during the final phase of rapid growth of the follicle involving, according to Corner, the last eight or ten hours preceding ovulation. This final spurt of growth in the follicle about to rupture could be associated with luteinization.

Our concept concerning the theca interna cells has changed. Corner (8) from studies on the rhesus monkey and Greulich (6) from observations of early human corpora lutea concluded that the theca interna cells contribute significantly to the formation of the corpus luteum and that they continue to add to it throughout its functional life. These cells as well as the granulosa cells probably produce progestin. It is entirely possible that the luteinization of the follicle may begin in these theca interna cells prior to follicle rupture and continue at a rapidly increased rate following ovulation. If this were so it would be possible to account for ovulation taking place with the rise in temperature rather than at its lowest point prior to the rise. Such a concept would explain fertile matings during the rise in the temperature curve. It would not be necessary to theorize an increased life span for

the ovum. Obviously, many variations can occur in a physiologic process as carefully timed as the reproductive function.

CONCLUSIONS

Additional experimental evidence has been presented that progesterone is responsible for the rise in body basal temperature during the luteal phase of the normal ovarian cycle in women. Ovulation in many, if not in most instances, occurs with the rise in the temperature rather than at its lowest point prior to the rise. It is probable that follicle luteinization begins in the theca interna cells during the stage of rapid growth just prior to ovulation and becomes accelerated with the rupture of the follicle. This explanation would account for the onset of the rise in body basal temperature prior to ovulation.

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A FLUOROMETRIC METHOD FOR THE CLINICAL DETERMINATION OF ESTRONE AND ESTRADIOL*

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INTRODUCTION

THE biological assay for estrogens based on the vaginal response in ovariectomized rats was first standardized by Kahnt and Doisy (1). This method has been used by many investigators for the estimation of estrogenic activity in various biological fluids. However, biological assay has the disadvantage of being long, expensive and subject to variations inherent in animals. It also necessitates the maintenance of a large animal colony.

Several investigators have proposed colorimetric chemical methods for estimating the estrogen content of urine in order to avoid biological assay (Kober (2), David (3), Talbot et al. (4), Szego and Samuels (5)). The urine of normal males and females contains such small concentrations of estrogens that the methods proposed usually lack sufficient sensitivity. This necessitates the use of large quantities of urine, so that nonspecific urinary chromogens interfere with accurate determination.

During the past several years, fluorometric methods have been perfected to determine minute amounts of thiamine, riboflavin (6) and some of the antimalarial drugs (quinacrine, quinine and chloroquine) (7). In the isolation and purification of estrone, Marrian (8) pointed out that when concentrated sulfuric acid is added to estrone, an orange color with greenish fluorescence is produced. Bierry and Gouzon (9, 10) attempted to use this fluorescent phenomenon for the qualitative detection of estriol in pregnancy urine. Slight modification of this reaction forms a color which is the basis of some of the colorimetric methods found in the literature (Kober (2)). While the present work was in progress, Finklestein, Hestrin and Koch (11) presented a quantitative fluorometric method for the estimation of crystalline estrone, estradiol and estriol. This method depends upon the formation of a fluorescent substance when phosphoric acid is added to dry

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[1] Part of this investigation was done during the tenure of a "Post War Residency of the Rockefeller Foundation."

crystals of the estrogens. However, the sensitivity is still not great enough for its general application without the use of large quantities of urine. Furthermore, the method had not been adapted to biological fluids.

A preliminary note on a fluorometric method for determining estradiol and estrone using the principle of Marrian has been presented (Jailer (12)). The great sensitivity of the method makes it possible to detect as little as 0.05 microgram of crystalline estrone. Data were offered to show its applicability to urine. Bates and Cohen (13) presented a somewhat similar method on pure crystalline natural estrogens, but gave no application of their method to the assay of biological fluids.[2]

The purpose of this communication is threefold; to present the analytical method in detail; to show evidence of its reliability and to present data on estrogen excretion in various normal and pathological conditions.

METHODS AND MATERIALS

Estrone, estradiol and estriol standards are dissolved in methyl or ethyl alcohol in the concentration of 10 milligrams per 100 milliliters. From these solutions, working standards containing 1.0 microgram per milliliter are made for estrone and estradiol and 4.0 micrograms per milliliter for estriol.

All organic solvents are redistilled before use, and all solutions are stored in glass stoppered bottles. The glassware must be scrupulously clean. Dow-Corning stopcock grease is employed. These precautions must be observed to prevent the appearance of nonspecific extraneous fluorescence.

For maximum fluorescence of the standards, 8.0 ml. of 60–70 per cent (by volume) sulfuric acid (Mallinckrodt, A. R., low nitrogen) are added to 0.2–0.5 ml. of alcohol containing various amounts of estrogens. This is stirred well with a rod and placed in a boiling water bath for exactly 5 minutes, cooled and the fluorescence read in a Coleman photofluorometer, Model #12. The B-2 and PC-9A filter combination is used. There is a quantitative relationship between steroid concentration and galvanometer reading (Fig. 1). For estrone and estradiol the range is between 0.05 and 0.25 microgram; however, the fluorescence of estriol is about one-twentieth that of the former two.

Several other steroids show this same reaction with sulfuric acid. β-estradiol fluoresces to the same extent as α-estradiol. Testosterone, progesterone and desoxycorticosterone fluoresce to a somewhat lesser degree. Cholesterol fluoresces very slightly, 20 micrograms giving the same fluores-

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[2] Bates and Cohen (1948) have now utilized this principle in the estimation of estrogen in pregnant mare urine (personal communication).

[3] Originally methyl alcohol was employed because of the greater solubility of the estrogens in it. However, it has been discovered recently that several batches of methyl alcohol, even after redistillation, contain substances which inhibit this fluorescence. No such phenomenon has occurred with redistilled ethyl alcohol.

cence as 0.05 microgram of estrone. The reaction has been performed with androsterone, dehydroandrosterone and pregnandiol in concentrations as high as 200 micrograms with no photoluminescence. These steroids have also been added to aliquots of urine which were analyzed by the method described below. Even though testosterone and progesterone fluoresce,

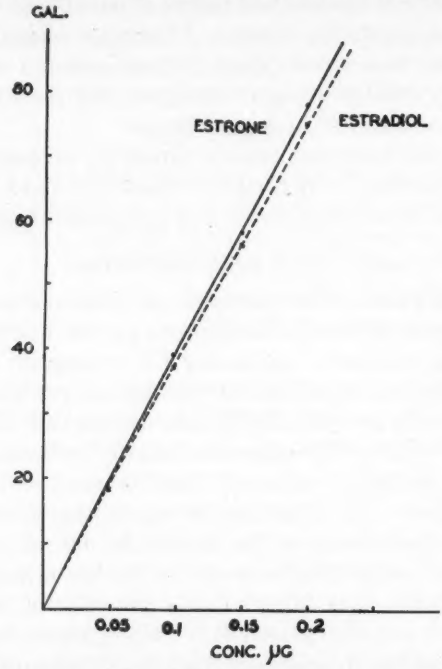

FIG. 1. Relationship between concentration of estrone and estradiol in micrograms and galvanometer readings (with B-2 and PC-9A filter combination).

when added to urine and extracted as described below, they do not interfere with the determination of estrogens. When 80 micrograms of desoxycorticosterone were added to urine there was some increase in the fluorescence, amounting to but a fraction of a microgram (Table 1).

Since the reaction with sulfuric acid is not completely specific, an extraction method was employed which separates the estrogens from other hormones which may also fluoresce. Use was made of the fact that the estrogens are soluble in alkali as contrasted to the other steroids mentioned, which are not. The method is a modification of the procedure of Bachman and Petit (14). It is as follows:

1. From 10 to 20 ml. of urine are hydrolyzed with 1 to 2 ml. of concentrated HCl, boiled for 7 to 10 minutes and cooled rapidly. (For pregnancy urine smaller aliquots are used.)

2. Transfer to a separatory funnel and saturate with NaCl.
3. Extract three times with benzene (A. R.; thiophene-free) using 25, 15 and 10 ml., respectively. (The interface gel should be saved as it contains some estrogen.)

TABLE 1. NONINTERFERENCE OF OTHER ADDED STEROIDS WITH THE FLUOROMETRIC METHOD

Urine (number)	Steroid Added	Amount (micrograms)	Galvanometer		Concentration of Estrogen in Aliquot (micrograms)
			B-2	B-1	
1	None	None	36	28	0.13
	Pregnandiol	200	40	29	0.14
2	None	None	21	12	0.07
2	Progesterone	200	23	14	0.07
3	None	None	75	35	0.27
3	Androsterone	100	73	33	0.26
3	None	None	76	35	0.28
3	Cholesterol	200	75	35	0.27
4	None	None	47	17	0.25
4	Desoxycorticosterone acetate	80	65	22	0.36
5	None	None	10	4	0.03
5	Testosterone	80	10	3	0.03

4. The combined benzene fractions are washed with distilled water, twice with 9 per cent Na_2CO_3, and again with water using 15 ml. for each washing. (This extracts the estriol.)
5. The benzene fraction is extracted four times with 2 normal NaOH using 20, 15, 10 and 10 ml., respectively.
6. The combined NaOH fractions are combined and acidified with 15 ml. of concentrated HCl.
7. Extract three times with benzene, 20, 15 and 15 ml.
8. The combined benzene fractions are washed with water, Na_2CO_3, and again with water, using 15 ml. for each washing.
9. The benzene is freed of water, and evaporated to dryness in a beaker.
10. 1.0 ml. of alcohol is added to the beaker.
11. A 0.7 ml. aliquot is transferred to a test tube and 8.0 ml. of 60 to 70 per cent H_2SO_4 added and stirred well with a fluted stirring rod. The tube is placed in a boiling water bath for 5 minutes.
12. Cool, transfer to cuvettes and read in photofluorometer.

In all determinations, it has been found necessary to run a reagent blank (dummy) consisting of 10 ml. of distilled water which is extracted in the

same manner. This is set at zero in the fluorometer. Recoveries (0.2 microgram and 0.5 microgram of estrone or estradiol) from water and urine are also run. These have been found to be fairly constant ranging between 50

TABLE 2. RECOVERY OF KNOWN AMOUNTS OF ESTRONE AND ESTRADIOL WHEN ADDED TO EITHER WATER OR URINE AND EXTRACTED AS DESCRIBED

(Micrograms Added)	Substance	Performed Estrogen	Total Estrogen	Estrogen Recovered	Per Cent Recovery
Estrone					
0.2	Water	0	0.104	0.104	52
0.2	Water	0	0.10	0.10	50
0.5	Water	0	0.32	0.32	64
0.5	Water	0	0.28	0.28	56
0.2	Urine	0.089	0.179	0.09	45
0.2	Urine	0.110	0.220	0.11	55
0.5	Urine	0.38	0.66	0.28	56
Estradiol					
0.2	Water	0	0.10	0.10	50
0.2	Water	0	0.12	0.12	60
0.2	Urine	0.27	0.38	0.11	55
0.2	Urine	0.26	0.36	0.10	50

and 60 per cent (Table 2). Standards of 0.2 microgram are set up at Step #11[4] and read against the absolute blank which consists of alcohol and sulfuric acid.

It has been noted that the color of the fluorescence of crystalline estrone and estradiol is yellowish green, whereas that of urinary extracts may show a whitish tinge as well. Therefore, an attempt was made to attain greater selectivity with various filter combinations. The maximum fluorescence with crystalline estrogens occurs with an exciting light of 435 millimicrons[5] (filter B-2). Changing the exciting wave length to 365 millimicrons (filter B-1) will decrease the galvanometer reading to practically zero (Table 3). However, the nonspecific fluorescence of the reagent blank is practically identical at the different wave lengths. It was also noted that the B-1 reading of urines containing very small amounts of estrogen is a much higher percentage of the B-2 reading than normal urines. Urines containing

[4] If necessary the sensitivity can be increased slightly by using sulfuric acid of higher concentration, e.g. 80 to 90 per cent. However, the concentration used should be well standardized.

[5] Since the B-1 filter contains a grid which allows 30 per cent transmission, a similar grid, of $\frac{1}{8}$ inch wire mesh, has been interposed in the B-2 filter. All values mentioned have been with this grid.

high amounts of estrogen, e.g. pregnancy urine or stallion urine, showed practically no fluorescence at 365 millimicrons. Thus, by reading the fluorescence of the urinary extract at the two wave lengths, one can determine the amount of estrogen and nonspecific fluorescence. The formula adopted for the correction factor is similar to the one proposed by Stimmel (15): $\frac{\text{Unknown } B_2 - B_1}{\text{Standard } B_2K(1-(B_1B_2)}$. B_2 is the galvanometer reading with the B-2 filter; and B_1, the reading with the B-1 filter; K is the constant of the ratio of the value of the reagent blank with the two filter systems. For these purposes it is unity.

For the biological assay of the estrogenic content of urine, the same extraction procedure as described above was employed, except for the stallion urines, where ether was used as the organic solvent. Girard separation was performed to separate the extract into ketonic (estrone) and nonketonic fractions (estradiol) in seven of the nine assays, and each assayed separately according to the method of Kahnt and Doisy (1). An aliquot was also saved and assayed for total estrogenic activity.

Twenty-four hour specimens were collected for the majority of cases; however, in some, accurately timed samples were analyzed. In most cases, completeness of collection was checked by creatinine determinations. Since recoveries of added amounts of estrone and estradiol averaged 50 per cent, it was assumed that recovery of the endogenous estrogens was similar; consequently all values reported in this communication have been computed on that basis and are twice the actual values obtained. This was not done when the chemical method was compared with biological assay; here it was assumed that the percentage recovery was identical in both.

Duplicate analyses of different aliquots of the same urine specimens have been made, on the same day and on different days The error was within 20 per cent.

The length of time necessary for the completion of a biological assay for estrogens depends upon the number of test animals available, but at best it takes several days. With the fluorometric method, using small aliquots of urine it is possible to analyze as many as five urines, together with a reagent blank and a recovery experiment, in one day.

RESULTS

After performing recovery experiments with estrone and estradiol from water and urine, data were accumulated to ascertain the reliability of this method.

1. *The Urinary Estrogen Excretion in Normal Women.* The urinary excretion of estrogens has been shown to be high in certain clinical conditions

TABLE 3

Substance	Galvanometer Reading		Per Cent Nonspecific Fluorescence
	B-2 435 millimicrons	B-1 365 millimicrons	
Estrone 0.2 micrograms	80	4	5
Estradiol 0.2 micrograms	76	5	7
Estriol 2.0 micrograms	40	2	5
Extract of urine from normal female	a) 56	35	63
	b) 95	50	56
	c) 87	44	51
Extract of urine from normal male	a) 70	29	42
	b) 78	37	48
	c) 40	20	50
Extract of urine from woman in 9th month of pregnancy	a) 25	0	—
	b) 54	9	17
	c) 46	0	—
Extract of stallion urine	a) 62	2	3.2
	b) 82	7	8.5
	c) 100	12	12
Extract of urine from case of craniopharyngioma	40	30	75
Extract of urine from woman past the menopause	16	10	75
Extract of urine from woman past the menopause	10	4	40
Extract of urine from woman past the menopause: (estrone added)	22	4	18
Reagent blank	a) 14	11	80
	b) 15	13	87
	c) 14	12	86
	d) 14	12	86
Nonpurified benzene	a) 32	32	100
	b) 87	94	108
Nonpurified ether	a) 25	21	84
	b) 14	13	93

and low in others, consequently an attempt was made to estimate the urinary excretion of estrogens by the fluorometric method and compare the results obtained with those reported in the literature.

The urine of two normal young women has been studied through three menstrual cycles by daily analyses for estrone and estradiol. The results are shown in Figure 2. It can be seen that there is a peak excretion at mid-interval and another just preceding menses. This is in agreement with results published by Gustavson *et al.* (16), Werner (17), Gallagher *et al.* (18) and others, as shown by bio-assay. These values were correlated with basal body temperature and it is shown that the mid-interval estrogen peak immediately precedes the rise in temperature. In one cycle, there was no abrupt rise in body temperature at mid-interval nor was there a sharp fall with menses; in this case there was no sharp peak of estrogen excretion as had been experienced during the preceding menstrual cycle (Fig. 2). In another menstrual cycle studied by daily urinary estrogen excretion, a "picket fence"-like graph was obtained, thus differing somewhat from the usual smooth graphs obtained by other investigators.

2. *Urinary Estrogen Excretion After the Administration of Estrogen.* It has been demonstrated by several investigators that the parenteral or oral administration of estrogens results in an increased excretion of these steroids in the urine. As shown in Table 4, two milligrams of estradiol benzoate were administered intramuscularly on two successive days to one normal young male (Case 1) and five milligrams of estrone sulfate were given orally on two successive days to another (Case 2). In addition five milligrams of estradiol in oil were given in single injections to each of two women in the menopause (Cases 3 and 4). Twenty-four hour specimens of urine were analyzed prior, during and after the administration of the estrogens. There was an increase in the urinary excretion of estrogens comparable with the results obtained by previous workers (Pincus and Pearlman (19); Heard and Hoffman (20)). The nonspecificity of the reaction suggested that the fluorometric method might determine degradation products of estrone and estradiol that are biologically inactive, consequently one of these urine specimens was analyzed by bio-assay as well. The results are compared in Table 5 (d) and it may be seen that they are in remarkably good agreement.

3. *Comparison of the Fluorometric Method with Biological Assay.* Urines from six patients were extracted according to the described method. Aliquots were taken for chemical analysis and the remainder assayed according to the method of Kahnt and Doisy. The correlation appears to be reasonably good considering the degree of error inherent in the biological method, the incompleteness of the Girard separation and the loss of biological activity in that procedure (Table 5).

The correlation between the bio-assay and the chemical method with the three samples of stallion urine was not as good as was the correlation in

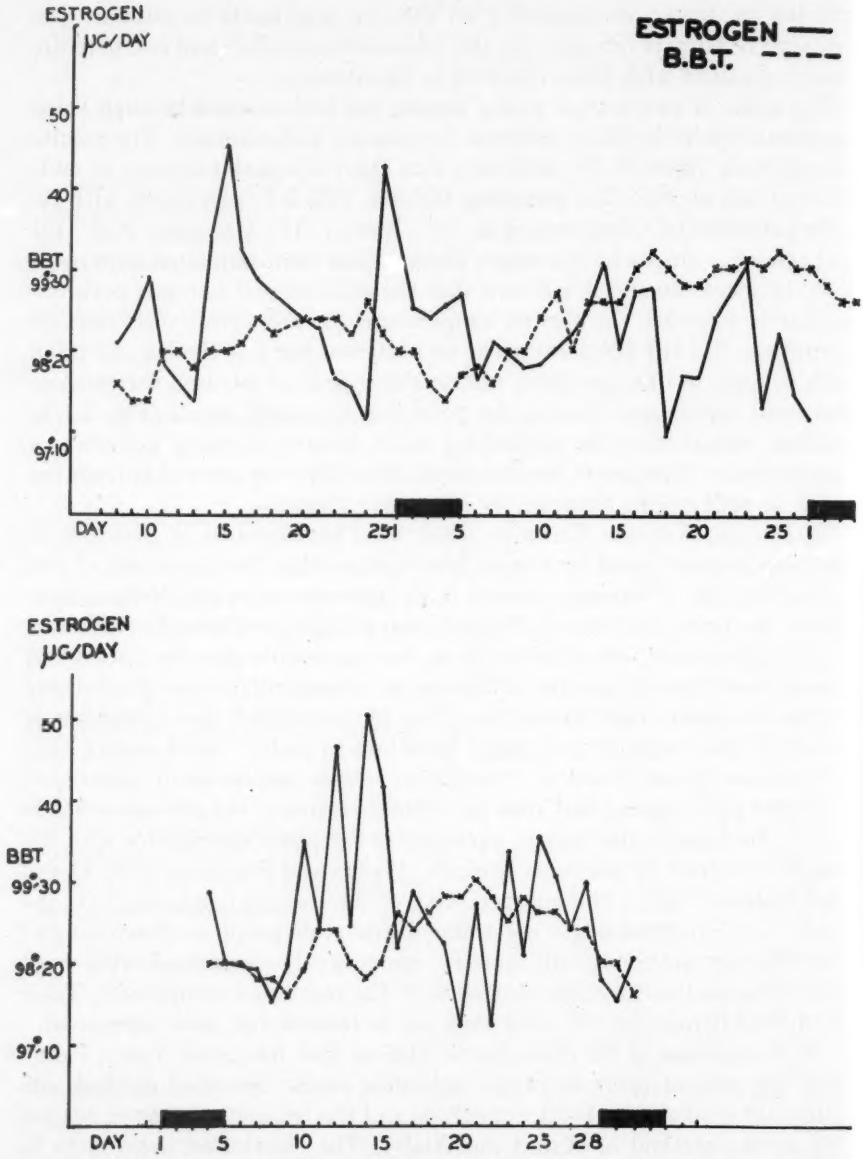

FIG. 2. Daily urinary excretion of estrogen (estrone and estradiol) in two normal young women. Blackened area represents menstruation. The unbroken line is estrogen excretion and the broken line is basal body temperature.

TABLE 4. THE EFFECT OF ADMINISTERED ESTROGEN ON THE EXCRETION OF FLUORESCENT SUBSTANCES IN THE URINE

	Case 1 (normal male)		Case 2 (normal male)		Case 3 (Two women in the menopause)	Case 4	
Day	Estrogen administered intramuscularly	Fluorescent substances in urine (micrograms)	Estrogen administered intramuscularly	Fluorescent substances in urine (micrograms)	Estrogen administered intramuscularly	Fluorescent substances in urine (micrograms)	
1		21.6		26.2		27.5	26.9
2	Estradiol benzoate (2 mg.)	19.2	Estrone sulfate (5 mg.)	88.0	Estradiol (5 mg.)	196.3	129.5
3	Estradiol benzoate (2 mg.)	46.7	Estrone sulfate (5 mg.)	116.0		62.2	47.1
4		52.0		61.7		41.4	29.0
5		70.3		23.2			

urine from human beings. There are several reasons for this discrepancy. Benzene was used as the organic solvent for the chemical method and ether was employed for extracting the estrogens for bio-assay.[6] The high concentration of estrogens in the extract necessitated great dilution of the final alcoholic extract in order to obtain a reading on the galvanometer scale. Thus, perhaps accuracy was impaired by the extreme dilution. These results are presented in Table 5.

4. *Estrogen Excretion in Various Normal and Pathological States.* The remainder of the data to be presented in this communication are concerned with the urinary excretion of total estrone and estradiol in various normal and pathological conditions. These are summarized in Table 6.

Normal males excrete between 20 and 26 micrograms of estrone within a 24-hour period. These values, obtained by the fluorometric method, are somewhat higher than those found by biological assay although values of this magnitude have been obtained occasionally (Gallagher et al. (18)). Children of both sexes excrete but small amounts. Women in the menopause excrete about the same amounts as women with amenorrhea, or

[6] I wish to thank Dr. Louis Levin for assaying the stallion urines and placing the results at my disposal.

TABLE 5. COMPARISON OF FLUOROMETRIC METHOD WITH BIOLOGICAL ASSAY, AS PERFORMED BY THE METHOD OF KAHNT AND DOISY

Urine from	Urinary Excretion of Estrogens						
	Fluorometric Method	Biological Assay					
		Total		Ketonic Fraction		Nonketonic Fraction	
	Micrograms	Micrograms	R.U.	Micrograms	R.U.	Micrograms	R.U.
Patient							
A.	23.1		20				
B.	12.4		20				
C.	220.0	128	490	94	94	34	280
D.	104.0	111	278	94	94	17	141
E.	136.0	114	400	84	84	30	250
F.	182.0	95	328	82	82	13	104
Stallion	*mg./liter*	*mg./liter*	*R.U./liter*	*mg./liter*	*R.U./liter*	*mg./liter*	*R.U./liter*
A.	10.00	29.1	52,000	26.0	26,000	3.1	26,000
B.	12.24	16.0	40,000	13.6	13,600	2.4	20,000
C.	7.74	11.08	20,800	10.0	10,000	1.08	9,000

normal women between peaks of excretion. Some of the older women, past the menopause, excrete much smaller amounts. One patient, aged 65, many years past the menopause, excreted no detectable estrogen. However, several women in this age group excreted as much as 20 to 30 micrograms per day.

Gravid women during the last trimester of pregnancy excrete large amounts of estrogen, as has been reported by many investigators (Zondek (21a), Smith and Smith (22)). These values obtained by chemical methods are lower than those obtained by bio-assay for comparable periods of gestation. It should be pointed out that estriol is not included in these results.

The highest values attained were in stallion urine, as described by Zondek (21b) and by Levin (23).

In only one case of gynecomastia was the estrogen excretion slightly elevated. This occurred in a 38-year-old male with typical "Klinefelter-Reifenstein-Albright syndrome" (24).

The urinary excretion was low in pituitary and adrenal hypofunction. It appeared normal in a young woman with Cushing's syndrome; but was exceedingly high in two children with virilizing adrenal tumors. In one, the urinary excretion of estrogens, after removal of the tumor, fell from the preoperative level of 114 micrograms to 6.6 and 4.4 micrograms per day.

DISCUSSION

A fluorometric method for the clinical estimation of estrogens (estrone plus estradiol) in urine has been presented. It eliminates the use of the biological assay. The great sensitivity of this fluorescent phenomenon makes it possible to assay urine containing physiological amounts of hormone using but small and convenient samples of urine. In standard solutions the range of estrogen concentration which can be determined in the Coleman fluorometer is between 0.05 and 0.25 microgram of estrone and estradiol. However, being a fluorometric method, it is open to various disadvantages of this general type of method. Cork and rubber stoppers must be avoided in order to prevent the appearance of extraneous fluorescence. New batches of reagents (especially methyl alcohol and sulfuric acid) must be checked before use, as it has been found that occasionally they may contain substances which inhibit fluorescence.

The lack of specificity is also a disadvantage. Other substances fluoresce but to a lesser degree. Finklestein *et al.* (11) and Bates and Cohen (13) have found that cholesterol does not fluoresce. It may very well be that the minute amount of fluorescence obtained with cholesterol in this study was due to a contaminant. It appears that the property of fluorescence depends upon the presence of an unsaturated bond in ring A. The substances which fluoresce, when treated with sulfuric acid, all seem to have this characteristic in common. Utilizing the differential solubility of the acidic estrogens in alkali, an attempt is made to exclude other steroids which may be found in urine. Therefore, we are actually determining lipoid-soluble phenols which, on the addition of sulfuric acid, fluoresce at 435 millimicrons and not at 365 millimicrons.

The phosphoric acid method of Finklestein *et al.* (11) does not have the great sensitivity of the sulfuric acid procedure. These workers have presented no data on the assay of urinary extracts. Bates and Cohen (13) have reported a similar method using 90 per cent sulfuric acid. The high concentration of the acid when added to urinary extracts may cause charring which colors the solution. The lack of sensitivity in their method appears to be due to their use of a filter system which does not give maximum transmission of the exciting light. Bates and Cohen (personal communication) have devised a very simple method for determining the amount of estrogen in the urine of pregnant mares, where the concentration of estrogens is high. The procedure using 90 per cent sulfuric acid does increase the sensitivity of estriol, so that with the proper filter system (B-2; PC 9A) it is ten times more sensitive than with 60 per cent sulfuric acid (unpublished data). Thus, with 90 per cent sulfuric acid, estriol has one-half the sensitivity of estrone or estradiol.

The data in this communication are presented with the view of showing the reliability of the fluorometric method. It has been demonstrated that

TABLE 6. URINARY ESTROGEN EXCRETION IN VARIOUS NORMAL AND PATHOLOGICAL STATES

Status	Number	Age (Years)	Remarks	Estrogen Excretion Micrograms/24 hours
Males	1	32	Normal	21.6
	2	31	Normal	26.2
	3	37	Normal	26.0
Children	1	10	Urethral stricture	6.5
	2	5	Cryptorchism	3.0
	3	5	Enuresis	4.2
	4	7	Hydrocele	5.3
Women during and past the menopause	1	54	Irregular and scanty menses	10.6; 26.6; 23.4
	2	50	Irregular and scanty menses	16.7; 13.7
	3	60	4 years past the menopause	5.9
	4	65	7 years past the menopause	0
Gynecomastia	1	27	No liver pathology	24.0
	2	54	Carcinoma of breast	17.3
	3	38	"Klinefelter" syndrome; 17-ketosteroids, 6.9; gonadotropins, 50 m.u.; small testes	32.8
Hypopituitarism	1	29	Prepuberal hypopituitarism	9.3
	2	41	Chromophobe adenoma	14.7
	3	41	Early Simmonds' disease	9.1
	4	40	Simmonds' disease	0; 0; 2.0
Adrenal disease	1	42	Classical Addison's disease, maintained on DCA	9.6
	2	27	Cushing's syndrome; adrenal hyperplasia 17-ketosteroids, 23 and 17 mg.	19.0
	3	7½	Virilizing tumor; 17-ketosteroids, 175 mg.	296.0
	4	6	Virilizing tumor; 17-ketosteroids, 189 mg.	114.0
			After removal of tumor	6.6; 4.4
"Primary ovarian agenesis"	1	18	Failure to grow; amenorrhea; minimal breast development; infantile uterus and genitalia	5.8

TABLE 6 (*continued*)

Status	Number	Age (Years)	Remarks	Estrogen Excretion Micrograms/24 hours
	2	17½	Height 4′10″; no breast development; amenorrhea; no pubic or axillary hair; infantile uterus: receiving stilbestrol	8.9
Ovarian tumor	1	64	Mass in adnexa; vagina and endometrium showed estrogenic stimulation	20.6
			Given x-ray treatment	4.8; 6.3
Pregnant women	1	20	8th month	365.0
			After zinc dust hydrolysis of specimen	549.2
	2	25	9th month	592.8
	3	30	10th month, 2 days before delivery	1500.0
Stallions	1		Normal	12240 (micrograms per liter)
	2		Normal	7740
	3		Normal	10000

the values obtained with this method are in the same order of magnitude as those reported in the literature in similar clinical conditions by biological assay. The administration of exogenous estrogens results in an increase in fluorescent substances in urine. Finally, satisfactory correlation between biological assay and the fluorometric method has been obtained in urines from six human subjects. The concentration of estrogens was so high in the urines from three stallions that serial dilutions of the urine for chemical analyses was necessary. This may have impaired the accuracy of the fluorometric analysis. Further comparison showed much better correlation between the two (unpublished data). This correlation is somewhat similar to that obtained between the capon test for androgen and the colorimetric method for 17-ketosteroids (Holtorff and Koch (25)).

Great difficulty has been encountered in applying this method to the determination of estriol in urine. It appears that the urinary chromogens cannot be separated from the estriol fraction by differential solubility. Difficulties thus far unsolved have also been met in attempting to adapt this method to blood and tissue. Szego and Roberts (26) have shown that

the estrogens are bound to proteins and that the usual protein precipitates do not free the estrogens from the protein binding. Thus, hydrolysis and extraction with organic solvents must be performed in the presence of proteins. However, work is in progress to apply this method to blood and tissue.

It appears that the values for the urinary excretion of estrogens obtained by the fluorometric method are somewhat higher than those reported in the literature with biological assay. It is especially so in urine from normal males and in urine from women in the menopause. This may be because some unknown nonestrogenic substances, in the acidic fraction, may fluoresce similarly to estrone and estradiol. Another possibility is that the filter system used may not be optimum, so that some of the nonspecific fluorescence is being recorded. The latter possibility is being investigated with an interference selective filter (524 millimicrons) which is close to the wave length of maximum transmission of the fluorescent light (Bates, personal communication).

CONCLUSIONS

1. Sulfuric acid in 60 to 70 per cent concentration, when added to alcoholic solutions of estrone and estradiol in the presence of heat, results in the development of a fluorescent compound.
2. A fluorometric method for the determination of estrone and estradiol in urine is presented, making use of this phenomenon.
3. Data in various normal and pathological conditions are made available to show the possible clinical application of the method.

ACKNOWLEDGMENT

The author wishes to express his appreciation to Dr. Earl T. Engle for his encouragement throughout the course of this work and to Mrs. Ruth S. Finkel for her technical assistance.

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EDWIN J. KEPLER
1894–1947

Obituary

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EDWIN J. KEPLER

1894–1947

Dr. E. J. Kepler was born in Erie, Pennsylvania, on January 22, 1894. He received the B.S. degree from Pennsylvania State College in 1916. He was an army bacteriologist in the first World War and, after the war, attended the University of Minnesota, where he received the M.D. degree in 1924 and where, also, he was admitted to the honorary medical society, Alpha Omega Alpha. For eighteen months he was an intern in Philadelaphia General Hospital. Thereafter, until his death, he lived and worked in Rochester, Minnesota, first as a Fellow of the Mayo Foundation, then as a Consultant in Medicine at the Mayo Clinic and as Professor of Medicine on the faculty of the Mayo Foundation, Graduate School, University of Minnesota. He was married to Dr. Helen A. Mackeen in 1921 and he is survived by her and their two daughters, Mary and Marcia.

These vital statistics are important, but they give no picture of Kep, as he was called by his many friends. Kep had so many facets to his nature that he is difficult to describe. He was known on the Mississippi as an enthusiastic riverman—one who liked to pilot his boat through seldom used channels, who enjoyed catching bass on flies and who was quite happy with the company of his family and friends. He was known at home as a family man who loved being at home. His friends think that Mrs. Kepler must have despaired at times at keeping order with all of the photographic and sporting equipment with which Kep surrounded himself. He was known in the Mayo Clinic as the most brilliant man in his section. He was a conscientious clinician and would spend any amount of time and effort in attempts, usually successful, to ferret out the diagnosis in difficult cases. Although his main interest was in metabolic diseases, his knowledge of general medicine was extremely wide. His contributions to clinical knowledge of diseases of the suprarenal glands were brilliant. It was in this field that he was best known by his friends outside Rochester.

When what he could write would serve a purpose, he wrote. He knew what he meant to say and his knowledge of English composition was such that he knew whether he had succeeded or failed in saying it. He did not release his material until it satisfied his knowledgeable standard.

He was a member of the American Association for the Study of Goiter (now the American Goiter Association), the Association for the Study of Internal Secretions, the Central Society for Clinical Research, the American Diabetes Association, the Laurentian Hormone Conference, the Central Interurban Clinical Club, and other organizations.

His death on October 19, 1947, from a coronary occlusion came as no surprise since his family, his friends and he himself were well aware of his serious heart condition. He is missed by his family, his colleagues and his many friends who, nevertheless, can rejoice in the fine influence which he created and which will always live.

EDWARD H. RYNEARSON, M.D.

THE ASSOCIATION

FOR THE STUDY OF

INTERNAL SECRETIONS

ABSTRACTS OF PAPERS PRESENTED AT THE THIRTIETH ANNUAL MEETING

FRIDAY AND SATURDAY, JUNE 18 AND 19, 1948

Palmer House, Chicago, Illinois

ABSTRACTS ARRANGED ACCORDING TO NUMBER ON PRINTED PROGRAM

FRIDAY, JUNE 18

9:30 A.M. *Red Lacquer Room*

1. PSEUDOGLANDULAR DISTURBANCES.

Hugo R. Rony. Chicago, Illinois.

In general, hormonal effects depend on end-organ sensitiveness as well as on the amount of the active hormone. Normal variations in skeletal growth, mammary development, amount of body hair, basal metabolic rate, etc., are probably due to variations in end-organ sensitiveness rather than to variations in the amount of hormones produced by the glands. Abnormal levels of end-organ sensitiveness may produce clinical pictures that closely imitate glandular disturbances—unresponsiveness resulting in pseudoglandular deficiency, hypersensitiveness resulting in pseudoglandular hyperactivity. Pseudoparathyroid deficiency, in this sense, has recently been described by Fuller Albright and his associates.

In the present paper attention is called to other types of abnormal end-organ response and to ways and means to differentiate them from true glandular disturbances. Cases of abnormal end-organ response are described that imitate thyroid, male and female gonadal, adrenal, insular and hypophyseal deficiency and hyperactivity. The likelihood of such origin is suggested for certain other types of "glandular" anomalies.

Recognition of the nature of these conditions is of considerable practical importance in that it may enable us to avoid errors in interpretation and disappointments in glandular therapy.

2. SYNDROME OF CRYPTORCHIDISM, HEART DISEASE AND NEVOID DERMATOSIS. REPORT OF TWO CASES.

S. J. Glass. Department of Endocrinology, Cedars of Lebanon Hospital, Los Angeles, Calif.

Two young adult, mentally alert males presented essentially the same multiple congenital anomalies, characterized by:

(1) unilateral cryptorchidism
(2) heart disease, well compensated in both subjects
(3) webbing of the neck and flaring of the ears
(4) inferior musculature
(5) hyperkeratotic-like dermatosis.

After the cure of the cryptorchidism by organotherapy in one subject and by surgery in the other, the sexual and skeletal growth continued uneventfully to relatively complete maturity.

3. Constitutional Precocious Puberty Controlled by Androgen Therapy.

S. Charles Freed and Minnie Goldberg. San Francisco, Calif.

A girl of four and one-half years had typical changes of precocious puberty. Investigations including laboratory determinations and x-rays seemed to indicate that the disorder was of the constitutional type. Methyltestosterone by mouth was administered for over two years and was successful in preventing menstruation and in suppressing the breast development. Bone age studies showed that while growth was markedly accelerated, the epiphyses of the long bones were nevertheless sufficiently well open to dispel fears of closure before a desirable height is attained. We have concluded that androgen therapy was beneficial in checking the psychologically traumatic features of precocious puberty as well as combating the serious threat of dwarfed stature usually associated with this condition.

4. A Study of the Biological Activity and the Magnitude of Endogenous Androgen Production in a Case of Adrenogenital Syndrome.

Anne C. Carter and Ephriam Shorr. From The Russell Sage Institute of Pathology and the Department of Medicine, Cornell University Medical College and The New York Hospital, New York City.

Although it is generally appreciated that, in virilism resulting from adrenal tumors or hyperplasia, urinary 17-ketosteroid values provide little information as to the amount or biological activity of their adrenal precursors, it has been difficult to set up experimental conditions in man by which the extent of endogenous hormone production could be quantitated. A case of virilism due to bilateral adrenal cortical hyperplasia in a 17-year-old girl provided conditions favorable for such an evaluation.

To this end a variety of histological and metabolic indices were selected which are influenced in opposite fashion by androgens and estrogens. These included vaginal smears, endometrial and skin biopsies, changes in the clitoris, breasts and rate of hair growth, urinary citric acid, calcium and creatine, and urinary hormone assays. Estrogens were then given in ascending doses until the effects of the endogenous androgens were neutralized, as judged by these criteria. Complete neutralization required 400,000 RU (40 mg. ethinyl estradiol, orally) of estrogen daily. On the basis of previous experiments in this laboratory on the neutralizing ratios of estrogens and androgens, it was estimated that this amount of estrogen would neutralize 3300 mg. of testosterone propionate given intramuscularly.

5. Pseudohermaphrodism: Early and Late Recognition.

M. James Whitelaw. Southern Medical College, Dallas, Texas, and St. Monica Hospital, Phoenix, Arizona.

Two children under five years of age are described showing the typical physical and chemical findings of male and female pseudohermaphrodites respectively, which included an elevated 17-ketosteroid excretion. At operation a hyperplastic adrenal was removed from each patient, following which their physical characteristics approached normal. Studies done on the total white count as well as the leucocyte-lymphocyte ratio previous to, and following operation showed no deviation from the normal. The third case is that of a 12-year-old dwarf female pseudohermaphrodite, who had been brought up as a boy. There was remarkable masculine physiognomy and physique. A 24-hour course of epinephrine was given to determine its effect on 17-ketosteroid output. There was some evidence of an increase. A plea is made for early recognition and surgical treatment of these cases.

6. Clinical, Laboratory, Operative, and Postmortem Observations in Infants and Children with Multiple Congenital Malformations (Turner's Syndrome, Ovarian Agenesis and Related Combinations).

Frank L. Plachte. From the Endocrine Clinic, Children's Hospital, Los Angeles, Calif.

A group of 15 infants and children with webbing of the neck and other congenital malformations has been under investigation. Their age ranges from the newborn period to late childhood. The group includes cases of Turner's syndrome, ovarian agenesis or related complexes of multiple congenital malformations and developmental retardation. Two of the patients are boys. Two of the group have also been studied at autopsy.

Several of the cases show unusual clinical and laboratory manifestations not previously reported in the literature. The urinary tract has been studied by pyelography, at operation, or at autopsy in thirteen of the group, and eight patients show urinary malformations of various types.

7. A Syndrome Characterized by Hypercalcemia, Calcinosis, and Renal Insufficiency Following Prolonged Intake of Calcium and Alkali.

Charles H. Burnett, Robert R. Commons (by invitation), Fuller Albright, and John E. Howard. Evans Memorial Hospital, Department of Medicine, Boston University School of Medicine, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston, and Johns Hopkins Hospital and University, Baltimore.

Data from six adult males have been collected. Each gave a history of prolonged periods (two to twenty-six years) of treatment for peptic ulcer or dyspepsia with large quantities of milk and absorbable alkalis. Pruritis or a complication of peptic ulcer were the usual reasons for hospitalization. At this time calcinosis, most frequently in the cornea or conjunctivae, was observed. Azotemia was a constant finding. Hypercalcemia without hypophosphatemia, and unaccompanied by hypercalcuria was demonstrated in five patients. Other less striking biochemical changes in the blood included moderately high serum carbon dioxide content and moderate hyperproteinemia without hyperglobulinemia. Evidence of severe renal disease was uniformly present; clearance measurements in three, demonstrated advanced insufficiency of all portions of the nephron. Two patients died and on one an autopsy was performed. Normal parathyroids and chronic pyelonephritis were demonstrated. In one of the four living patients parathyroid hyperplasia was demonstrated at operation. In another a normal kidney biopsy was obtained two years before renal failure occurred. Low calcium, low alkali regimens have produced symptomatic improvement and decreased serum calcium, nonprotein nitrogen, and carbon dioxide content.

8. Hypoparathyroidism, with Mental Troubles and Ectodermal Disorders.

Manuel Villaverde. Habana, Cuba.

Idiopathic latent tetany is a rare condition. Nevertheless, the syndrome outlined in this paper is frequent (0.6% to 1% of the total consultations in the office). It is of hypoparathyroid nature, as proved by the clinical picture of latent tetany, the nervous irritability, the results of blood chemistry, the family endocrine background, and the impressive improvement of the patients during adequate therapy. These elements sustain the diagnosis of hypoparathyroidism in patients suffering from mental troubles frequently accompanied by ectodermal disorders, namely, decalcified and abraded teeth, and early appearance of gray hair. The nervous symptoms range from a troublesome nervousness, which we may call cycloid or schizoid, to anxiety, compulsive neurosis,

mania, crises of weeping, circular insanity, and sometimes schizophrenia. Patients may give a history of spasmophilia, cramps, or true tetanic crises. The gray hair, which is not different from common gray hair, appears before the thirties. The teeth become sensitive, and show decalcification and abrasion of the free borders; minute fractures sharpen the teeth making them look like a saw, and their borders appears translucent and bluish. Sometimes crises of greater sensitivity may announce an increase of the destructive process, which can shorten the teeth to half or less of their former size.

These three complaints—mental troubles, abraded teeth, and gray hair before the thirties—are the elements that constitute the syndrome, the study of which is to be encouraged because of its frequency, and the excellent results of therapy. Hyperestrogenism is often the cause of the syndrome. There are some cases with predominant mental symptoms.

9. Treatment of Far-Advanced Inoperable Carcinoma of the Breast with Estrogens and Androgens.

Samuel G. Taylor, III, Danely Slaughter, Frederick W. Preston. Chicago, Illinois.

Clinical results in the treatment of advanced inoperable carcinoma of the breast with various estrogens and androgens will be reported. The series includes 44 patients who were treated with estrogens and 30 patients treated with androgens over a two-year period.

The most favorable results with estrogen therapy were obtained in the older age group. Reduction in the primary tumor, in regional lymph gland metastases and in recurrences in the skin, with healing of ulceration occurred in some patients. However, there was occasional acceleration in tumor growth. In no patient given estrogen therapy were we able to demonstrate regression of osseous metastases, but in several patients there was no progression in these metastases during observation.

Androgen therapy produced regression in soft tissue metastases in several patients: however, improvement in osseous lesions was more frequent. Severe masculinization commonly occurred and six patients developed congestive heart failure requiring omission of androgen therapy.

In one patient a large carcinomatous ulceration disappeared during estrogen therapy but recurred in nine months. This did not respond to estrogen therapy a second time but regressed during androgen therapy.

A few patients were given desoxycorticosterone. No regression in the tumor was noted during this therapy.

We have not been able to predict whether estrogen will prove to be more efficacious than androgen therapy in any individual case. Estrogens are not ordinarily of value in the younger age group.

10. Hormonal Factors Involved in the Regulation of Basal Body Temperature During the Menstrual Cycle and Pregnancy.

Charles L. Buxton and William B. Atkinson. Departments of Obstetrics and Gynecology and of Anatomy, College of Physicians and Surgeons, Columbia University, N. Y., and the Sloane Hospital for Women, N. Y.

In order to ascertain the cause of the well known postovulatory rise in basal body temperature in normal cyclic women, six patients with amenorrhea and with little or no endometrial activity were given sequential therapy of estrogen followed by progesterone. The patients were followed by means of temperature charts and endometrial biopsy. The basal body temperature invariably rose significantly (three-fifths to one degree F.) during progesterone therapy.

The postovulatory temperature rise was also maintained and menstruation postponed ten to thirty days in normal women by the administration of chorionic gonadotropin. However, chorionic gonadotropin administered to a castrate following estrogen priming did not result in any significant change in basal temperature. It was assumed, therefore, that the maintenance of the postovulatory temperature rise by chorionic gonadotropin was due to its luteotropic effect and that here also progesterone produced the temperature response.

In order to determine whether the rise were maintained throughout gestation, three women (one through two pregnancies) kept temperature charts until delivery. One other is now in the sixth month. The temperature fell to its preovulatory level uniformly between the fourth and fifth month in all cases. This is in spite of the commonly supposed steady rise in progesterone activity as determined by pregnandiol excretion. Some conjecture, but no final explanation is offered for this interesting phenomenon.

11. The Effects of Certain Steroids—Intramuscular and Sublingual—on the Basal Body Temperature of the Adult Human Male.

Robert M. Perlman. San Francisco, Calif.

Fifteen adult human males, exhibiting different degrees of prostatism and/or the male climacteric received various doses of androgens, estrins, and progestins, intramuscularly and sublingually, during a period of ten months. Relative effects on the earliest waking rectal temperature—or basal body temperature (B.B.T.)—were noted.

The average B.B.T. of males restored to well being with androgens was stabilized usually in the range of 98° F ±0.2° F. Massive dosage enhanced this effect. In several therapy-resistant cases, placation was obtained with extraordinary i.m. testosterone propionate doses of 150–200 mg. three times weekly.

In five cases, due to prohibitive costs of massive androgen therapy, estrins were substituted. A single dose of 2.5–5.0 mg. of equine estrogenic substances ameliorated symptoms of prostatism and, following an initial temperature lag of one to two days, depressed and maintained the B.B.T. at approximately 97.5° F to 97.8° F for one to four weeks. Penile erections were suppressed. No breast changes were observed grossly during three to four months.

Progesterone, 10–50 mg. i.m., produced B.B.T. elevations ranging from 0.1° F to 2.0° F. "Linguets" of anhydrohydroxyprogesterone acted similarly; *however*, doses 10–30 times greater, mg. for mg., were required to produce equal effects. Sensitivity to progestin fluctuated markedly from individual to individual. *In the same individual, graduated doses initiated thermal elevations directly proportional to the size of the dose.* Saturation doses gave temperature levels of 98.4° F to 99° F. Thermal rises persisted, in most cases, five to seven days, declining progressively, dipping, and returning to approximate starting points. *Administration of progestin resulted in intense recrudescence of prostatic and/or climacteric symptomatology.*

2:00 P.M. ***Red Lacquer Room***

12. A Simplified Hypophysectomized Rat Adrenal Ascorbic Acid Bioassay Method for Adrenocorticotropin (ACTH): Specificity and Application to Preparative Problems.

Paul L. Munson, Alfred G. Barry, Jr. (by invitation), and F. C. Koch. From the Pituitary Research Section, Biochemical Research Department, Armour and Company, Chicago.

The method of Sayers, Sayers, and Woodbury (*Endocrinology*, in press) for bioassay of adrenocorticotropin (ACTH) in the 24-hour-hypophysectomized rat involves com-

parison of the control ascorbic acid concentration determined in one adrenal removed just prior to intravenous administration of hormone with that of the second adrenal removed one hour later. The decrease is proportional to the amount and potency of the preparation administered. In the present modification, both adrenals are removed one hour after injection and analyzed together. This and other simplifications result in a saving of over half the assay labor without sacrifice of accuracy and have permitted application of the method to over 8000 animals. Using 20 to 30 rats for controls, Armour ACTH Standard and "unknown," the potency of the latter can be estimated with a standard error of 20 to 30 per cent.

The assay method has aided in the preparation of gonadotropic, lactogenic, thyrotropic, and growth hormone preparations essentially free from ACTH, incidentally providing evidence that these hormones do not interfere in the assay.

The recovery of the ACTH potency of a pituitary preparation in its sub-fractions, and the variations found following differing treatments to reduce oxytocic activity illustrate the usefulness of the method in ACTH preparative problems.

13. Content of Adrenocorticotropic Hormone (ACTH) in the Rat Pituitary under Optimal and Stressful Environmental Conditions.

George Sayers, Marshal Kerkin (by invitation) and J. N. Tortoreto (by invitation). From the Department of Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah.

A method has been developed for the quantitative determination of the ACTH content of rat pituitaries. Lyophilized anterior pituitary tissue is extracted with 0.01 N sodium hydroxide in 0.9 per cent sodium chloride. The extracts are assayed for ACTH by the adrenal-ascorbic acid depletion method. Each pituitary (dry weight equal to one milligram) from rats kept under optimal environmental conditions has a biological activity approximately equivalent to 0.1 milligram of highly purified preparation of porcine ACTH (62AA).

Pituitaries from animals injected with histamine (10 milligrams per 100 grams of body weight) and sacrificed at the end of one hour have a biological activity equivalent to 0.07 ± 0.01 milligram of 62AA.

The adrenals of these histamine-injected animals show a depletion of ascorbic acid which can be produced by the administration of 0.05 milligram of ACTH (62AA). Thus the amount of ACTH released, as measured by the effect on adrenal ascorbic acid is largely accounted for by the loss of tropic activity from the pituitary tissue.

These data indicate that sufficiently large quantities of ACTH are stored in the pituitary to take care of the immediate requirements of the animal during the response to acute stress. Further work is in progress to determine the rate of elaboration and synthesis of this tropic hormone by the pituitary under a number of other experimental conditions.

14. The Activation of the Adrenal Cortex by Insulin Hypoglycemia.

H. Gershberg (by invitation) and C. N. H. Long. Department of Physiological Chemistry, Yale University, New Haven, Conn.

Cannon, McIver and Bliss have shown that insulin hypoglycemia causes activation of the autonomic nervous system and release of epinephrine. Since it has recently been shown that epinephrine also brings about release of the adrenotropic hormone of the pituitary and activation of the adrenal cortex, experiments have been carried out to test the hypothesis that the physiological counteractivity to insulin hypoglycemia is a

consequence of the combined action of both components of the adrenal gland. Rats were injected with 1 unit of insulin per kilo of body weight and the adrenal ascorbic acid and blood glucose were determined 1 hour later. It was found that if the blood glucose fell below 40 mg. per cent, there also occurred a large decrease in adrenal ascorbic acid, an effect which is now known to be due to the release of adrenotropic hormone.

However, if glucose is given along with the insulin to prevent a fall in the blood glucose, then no loss of adrenal ascorbic acid was observed. That it is not the level of blood glucose itself but the release of epinephrine that determines the activity of the pituitary is shown by the fact that the administration of glucose does not prevent the activation of the pituitary in such situations as exposure to cold or trauma, where the release of epinephrine is due to cause other than hypoglycemia.

15. Influence of Adenotropic Hormone on Sodium Excretion in Hypophysectomized Rats.

Betty L. Rubin and Ralph I. Dorfman. From the Departments of Biochemistry and Medicine, Western Reserve University School of Medicine and Lakeside Hospital, Cleveland 6, Ohio.

The influence of adrenotropic hormone on sodium excretion in hypophysectomized male rats has been studied using a method previously described (Dorfman, Potts, and Feil; *Endocrinology* **41**: 464, 1947). This method employs radiosodium and has been shown to detect as little as one microgram of desoxycorticosterone.

Two preparations of adrenotropic hormone have been used. A highly purified preparation, kindly supplied by Dr. C. H. Li, produced statistically significant increases in sodium excretion during a six-hour test period. Under similar conditions a crude preparation, kindly supplied by Dr. D. A. McGinty, gave less consistent results, but sodium retention was indicated. The significance of these findings will be discussed.

16. Factors Influencing the Corticotropin Production of the Anterior Pituitary.

Hans Selye. From the Institut de Medecine et de Chirurgie experimentales, Universite de Montreal, Montreal, Canada.

Earlier experiments lead us to believe that under the influence of nonspecific stress (e.g., cold, infections, intoxications, nervous stimuli), corticotropin production by the pituitary is increased and this secondarily causes an excessive production of corticoids (to raise the resistance against nonspecific stress) as part of the "General-Adaptation-Syndrome." Sometimes, such prolonged stress also leads to hypertension and nephrosclerosis especially in animals sensitized by unilateral nephrectomy and diets rich in sodium and protein. It was striking, however, that while anterior-pituitary preparations or synthetic corticoids (e.g., desoxycorticosterone acetate) regularly cause nephrosclerosis and hypertension in animals so sensitized, stress (e.g., cold) did so only in a comparatively small percentage of cases. In clinical medicine stress appears to be a factor in the production of nephrosclerosis and hypertension, yet, usually in patients exposed to the continuous stress of wasting diseases, hypertension does not ensue and the adrenal cortex is not always unusually large.

Recent experiments in the rat have thrown some light upon these questions; they indicate that in the rat, stress (e.g., cold) causes marked adrenal cortical enlargement only when the protein intake is high (30% casein diets) and not when it is comparatively low (15% casein diets). A synthetic diet containing protein hydrolysates or essential amino-acids (in a quantity equivalent in nitrogen to a 30% casein diet) permits the same stress-hypertrophy of the adrenal as the high-protein diet. Apparently, amino-acids

are necessary for the normal corticotropin production during stress. It must be pointed out that high-protein diets in themselves did not cause adrenal enlargement, nephrosclerosis and hypertension under our experimental conditions so that apparently both protein catabolism and stress are necessary in order to insure optimal corticotropin production. In rats in which sudden protein catabolism is elicited by starvation, thyroxin, cold and other stresses, the adrenal cortex undergoes marked enlargement, especially if the animals were previously kept on high-protein diets and thus allowed to store protein. Conversely, after prolonged feeding on low-protein diets the adrenal response to stress is slight.

These observations suggest that in clinical medicine, hypertrophy of the adrenal and the subsequent development of "diseases of adaptation" due to corticoid intoxication may likewise depend upon simultaneous changes in protein metabolism induced by the stress.

17. The Use of Adrenocorticotropin as a Test of Adrenal Cortical Reserve.

George W. Thorn, Peter H. Forsham (by invitation), Lillian Recant (by invitation), and A. Gorman Hills (by invitation). Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

In man ACTH has been shown to be capable of stimulating the secretion of all known types of physiologically active adrenal cortical steroids. In a large group of normal subjects and patients without evidence of adrenal cortical insufficiency a fall in circulating eosinophils and a rise in urinary uric acid/creatinine ratio (the change exceeding 50 per cent in both instances) was observed following a single dose of 25 mg. of ACTH injected intramuscularly. Thirty-five patients with classical Addison's disease showed a small or insignificant fall in circulating eosinophils (mean value 6 per cent) and a rise in urinary uric acid/creatinine ratio (mean value 24 per cent). There was no overlap between the control group and the Addisonian group with respect to the changes in circulating eosinophils. There was some overlap between the maximal increase in uric acid/creatinine ratio observed in the less severe Addisonian patients and the poorest response of apparently non-Addisonian patients. A group of patients, complaining of weakness, fatigue, and hypotension without other evidences of adrenal cortical insufficiency and with evident psychological and emotional disturbances, showed an excellent response to the ACTH test dose. Hence primary adrenal cortical insufficiency could be readily excluded in this group by a relatively simple test which required only four to six hours for completion. Eight patients with evidence of anterior pituitary deficiency failed to show an entirely normal response to a single test dose of ACTH but did show some response to a forty-eight hour period of continuous administration, i.e. 10 mg. every six hours.

Patients with Cushing's disease revealed an extremely low initial level of circulating eosinophils (2 to 20 cells per cu. mm.) in contrast to normal subjects (100 to 300 cells per cu. mm.). The initial fasting urinary uric acid/creatinine ratio was similar to the values obtained in normals following ACTH administration.

18. Observations on the Pituitary-Adrenal Response Following Epinephrine Infusion in Man.

Lillian Recant (by invitation), Peter H. Forsham (by invitation), and George W. Thorn. Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

Long and his co-workers have demonstrated the response of the adrenal cortex to the administration of epinephrine to rats with an intact anterior pituitary. In previous

studies the authors have demonstrated that a sudden increase in 11–17-adrenal oxysteroids is followed by a rapid fall in circulating eosinophils in both man and rats under control conditions.

Employing the fall in circulating eosinophils as an index of adrenal steroid response, normal subjects, patients with Addison's disease, and patients with pituitary insufficiency have been investigated following the intravenous administration of 1.5 mg. of epinephrine hydrochloride in 200 cc. of saline over a one-hour period. Normal subjects and patients without evidence of adrenal or pituitary insufficiency show a maximal drop in eosinophils of 70 to 80 per cent in four hours and a maximal lymphocyte fall of 30 per cent in two hours. Patients with pituitary insufficiency show practically no change in circulating eosinophils. Patients with Addison's disease show a definite but variable response which correlates approximately with the severity of the Addison's disease clinically. These studies suggest that in man the secretion of pituitary ACTH is stimulated by epinephrine and that it is possible by this technique to differentiate between primary adrenal and pituitary-adrenal insufficiency. Studies in rats confirm these observations.

19. Fate and Metabolic Action of Intravenously Administered Adrenocorticotropic Hormone (ACTH).

Thomas W. Burns (by invitation), George Sayers, Frank H. Tyler (by invitation), B. V. Jager (by invitation), T. B. Schwartz (by invitation), Emil L. Smith (by invitation) and L. T. Samuels. From the Departments of Pharmacology, Medicine and Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah.

A normal male subject was infused with 200 ml. of 0.85 per cent sodium chloride containing 240 mg. of A.C.T.H. (Armour's 37-KE, containing 0.005 units of pressor activity per mg.) over a period of 50 minutes. Except for a mild chill which lasted 25 minutes no untoward reactions occurred. Blood pressure remained within normal limits except for a slight rise during the course of the chill. At the end of the infusion the plasma level of A.C.T.H. (as bioassayed by the adrenal-ascorbic acid depletion method) was approximately 3000 micrograms per 100 ml. plasma; within the first hour after infusion the level had dropped to 200 micrograms per 100 ml., and at the end of 5 hours was about 25 micrograms per 100 ml. There was no detectable activity in the plasma at the end of 24 hours. Less than 200 micrograms was excreted in the urine during this 24-hour period.

Following hormone administration, urinary 17-ketosteroids and neutral-lipide reducing substances (Heard, Sobel and Venning) were elevated. A transitory mild elevation in the fasting blood glucose occurred. A diabetic type of response was obtained from a meal ingested 6 hours after hormone administration. The level of serum phosphate fell, suggesting glycogen storage. These changes occurred concomitantly with an increase in urinary nitrogen excretion. The serum uric acid level remained unchanged while uric acid excretion was increased. There was a slight but apparently significant increase in the creatinine clearance. Circulating eosinophiles and lymphocytes were greatly reduced in number. No changes took place in plasma albumin and gamma globulins as determined by salt fractionation and electrophoretic methods. Anti-streptolysin "O" and anti-typhoid antibodies failed to rise. Certain serum peptidases were elevated. Blood pH, serially determined, reached a maximum level of 7.60. All of the above metabolic changes reached a maximum and had returned to normal within the first 12 hours after A.C.T.H. administration.

20. Metabolic Changes Following the Administration of Pituitary Adrenocorticotropic Hormone (A.C.T.H.) to Normal Humans.

H. T. McAlpine (by invitation), E. H. Venning, L. Johnson (by invitation), V. Schenker (by invitation), M. M. Hoffman and J. S. L. Browne. McGill University Clinic, Royal Victoria Hospital, Montreal, Quebec.

Massive doses (240 to 420 mg.)* of adrenocorticotropic hormone (Armour) were given to two normal males and one normal female in divided doses over a twenty-four hour period. The subjects were maintained on a constant diet for a control period and throughout the experiment. Following the administration of the hormone there was a decreased urinary excretion of sodium and chloride and an increased excretion of potassium, uric acid and nitrogen. This was followed by a sodium and chloride diuresis and a decreased excretion of potassium. In addition, following the A.C.T.H. administration there was a striking urinary excretion of glucose, varying from 12 to 30 grams. In spite of this glycosuria there was a marked reduction in the urine volume (in one case to 600 cc. per day). There was also a moderate elevation of the blood sugar. The slight increase in nitrogen excretion was insufficient to account for the amount of glucose excreted. The significance of these findings with regard to the pituitary-adenal relationship will be discussed.

21. The Effect of Adrenocorticotropin on Antibody Levels in Normal Human Subjects.

P. H. Herbert and J. A. de Vries (introduced by J. S. L. Browne). McGill University Clinic, Royal Victoria Hospital and Department of Bacteriology and Immunology, McGill University, Montreal.

Subjects were immunized with a commercial preparation of staphylococcal toxoid and later received corticotropin. Before the experiments were initiated the anti-hemolysin titres were allowed to fall well below the maximum previously attained. Figures for antibody levels before and after injecting the hormone are presented, together with total, eosinophil and differential leucocyte counts and plasma protein and hematocrit values.

The quantities of hormone injected ranged from 40 to 420 mg. (equivalent to 28 to 210 mg. of the Armour standard preparation LA-1-A) the smaller amounts being given in a single injection and the larger in a series of injections over periods varying from one hour to twenty-four hours.

In no subject was there a significant rise in antibody level after giving corticotropin even though the larger doses were sufficient to cause a great increase in the excretion of urinary corticoids. In all subjects there was a fall in the lymphocyte count, the least being 25% and the greatest 68% of the initial control value. These lymphocyte counts returned quickly to normal and the extent of the fall was not related to the amount of hormone given. The eosinophil counts fell more quickly than the lymphocyte counts and the decrease was greater with the larger amounts of hormone. In experiments in which injections were continued for the longer periods these cells almost disappeared from the blood. A fall in hematocrit of 10–17% suggested an increase of blood volume but the plasma proteins in most cases tended to maintain the initial levels.

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* These amounts are equivalent to 120 to 210 mg. of the Armour standard preparation LA-1-A.

22. A Comparison of the Effect on Bone Formation of the Hyperadrenocorticism of Cushing's Syndrome with That Induced by Adrenocorticotropic Hormone (A.C.T.H.).*

Frederic C. Bartter, Anne P. Forbes and Fuller Albright. Massachusetts General Hospital, Boston, Mass.

One of the cardinal features of Cushing's syndrome is osteoporosis. By definition, osteoporosis is that form of demineralization which is due to decreased formation of bone matrix by osteoblasts. The serum alkaline phosphatase, an index of osteoblastic activity, is low. It is thus a disorder of protein metabolism, not of calcium metabolism. However, one cannot deposit calcium without matrix. It follows that the development of osteoporosis will be accompanied by an increased excretion of calcium if bone destruction remains constant.

The data to be presented are taken from metabolic balance studies on patients with panhypopituitarism, acromegaly, osteitis deformans, and ovarian agenesis. The administration of A.C.T.H. produced a marked increase in urinary calcium excretion in all patients, save two who received stilbestrol throughout the study. In the three patients in whom the serum phosphatase was initially elevated, A.C.T.H. produced a fall; in two of these the hypercalcuria was accentuated.

These studies support the hypothesis that A.C.T.H. causes elaboration of an adrenal cortical hormone which inhibits the production of bone matrix by osteoblasts.

23. Adrenal Cortical Unresponsiveness in Patients with Gastric Cancer.

Edward C. Reifenstein, Jr., N. F. Young, Aurelia Potor, Benedict Duffy and F. Homburger. Department of Clinical Investigation of the Sloan-Kettering Institute, Memorial Cancer Center, New York.

Persons with normally responsive adrenal cortices respond to the injection of anterior pituitary adrenocorticotropic hormone with a fall in the blood eosinophil level and a rise in the urinary uric acid/creatinine ratio. In contrast, the majority of patients with gastric cancer respond to injection of this hormone preparation only with the fall in the eosinophil level. Studies are in progress to determine the nature of the lack of response to pituitary tropic hormone stimulation of the adrenal cortical mechanism for regulating the uric acid/creatinine ratio in patients with gastric cancer.

Previous studies have shown that the administration of adrenal cortical extract is necessary to induce normal liver glycogen deposition in patients with gastric cancer. The effect of the administration of adrenocorticotropic hormone on liver glycogen deposition in patients with gastric neoplasms is being studied to see whether in patients with this disease the adrenal cortical mechanism involved in glycogen deposition is also unresponsive to pituitary tropic hormone stimulation.

It remains to be determined whether the adrenal cortex does not respond to pituitary tropic hormone stimulation because it is less sensitive or because it is functioning to the limit of its capacity.

24. The Excretion of Adrenal Metabolites in Human Urine.

Konrad Dobriner, Seymour Lieberman and C. P. Rhoads. Sloan-Kettering Institute for Cancer Research, New York.

Androsterone and 11-hydroxy androsterone have been recognized as normal constituents in the urine of males and females. In the course of hydrolysis 11β-hydroxy andros-

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* The A.C.T.H. was furnished by Dr. John Mote of the Armour Laboratories and by Dr. Choh Hao Li of the University of California.

terone is extensively converted to the corresponding unsaturated compound, Δ^9 androstenol-3α-one-17. This unsaturated substance is difficult to separate from androsterone and the values reported for "androsterone" unquestionably include variable amounts of the unsaturated analogue. With the aid of infrared spectrometry, we have been able to separate the Δ^9-steroid from androsterone. The amount of Δ^9-androstenolone present in normal urine is variable but is generally somewhat less than the amount of androsterone. In adrenal hyperplasia of the virilizing type, several investigators have claimed that the excretion of androsterone is at an elevated level. By means of our procedures, we have been able to establish that this apparent increase in androsterone excretion can be attributed largely to the presence of considerable amounts of Δ^9-androstenol-3α-one-17. The large "androgene" excretion in adrenal hyperplasia is the consequence of increased production of adrenal cortical hormones rather than excessive gonadal activity.

In these same individuals the excretion of etiocholanolone is at a somewhat elevated level which adds further confirmation to the view that at least a part of this substance is of adrenal origin. The corresponding 11β-hydroxy etiocholanolone which is recognized likewise as its dehydration product, Δ^9-etiocholenol-3α-one-17 is rarely found in the urine of normal persons and has as yet not been found in the urine of patients with adrenal hyperplasia. This substance, is, however found in a significant number of patients with neoplastic disease. The excretion of these and related substances in patients with adrenal tumors and in Cushing's syndrome will also be discussed.

SATURDAY, JUNE 19

9:00 A.M. ***Red Lacquer Room***

25. PREPARATION OF CRYSTALLINE GROWTH HORMONE.*

Jacob B. Fishman (by invitation), Alfred E. Wilhelmi (by invitation) and Jane A. Russell. Department of Physiological Chemistry, Yale University, New Haven.

In a preliminary note (*Science*. **106**: 402, 1947) it was reported that crude crystalline preparations of growth hormone comparing favorably in activity with the pure hormone can be produced easily in good yields by a simple ethanol fractionation of a calcium hydroxide extract of fresh beef anterior pituitary glands. The crude fractions are electrophoretically heterogeneous and contain, in addition to their growth activity, a small but significant amount of thyrotropic activity and a high degree of glycostatic hormone activity. Little or none of the other major types of pituitary hormone activity is present. Further purification of the crude crystalline material has been achieved as follows: The material is dissolved in 0.1 M potassium chloride with the aid of potassium hydroxide to make a 0.5 per cent solution of pH 11.0. The solution is adjusted to pH 5.0 with hydrochloric acid and the precipitate that forms is centrifuged off. The supernatant solution is adjusted to pH 8.5–8.7 and one-fifth volume of 95 per cent ethanol is added dropwise to the rapidly stirred solution. The crystalline precipitate that forms is easily centrifuged off, the crystals are suspended in distilled water, dialyzed until the outside solution is free of chloride, and lyophilized. The product so obtained is nearly homogeneous upon electrophoretic examination. Two such preparations, injected into hypophysectomized male rats of about 100 grams body weight at the rate of 20 micrograms per day for 10 days brought about growth increments of 13.3 and 15 grams. The purified material still has

* The work here reported is being done under a grant from the American Cancer Society on the recommendation of the Committee on Growth, National Research Council.

high glycostatic activity. Additional details of the biological, chemical and physical properties of the material will be reported. Although this crystalline product is not quite pure, the ease and simplicity of the preparation and the excellent yields—of the order of 1 gram per Kg. of fresh gland—should make possible rapid advances in both the experimental and the clinical study of the properties of the growth hormone.

26. The Influence of Purified Growth Hormone on Fasting Metabolism.*

Clara M. Szego and Abraham White. From the Department of Physiological Chemistry, Yale University, New Haven, Conn..

A highly purified growth hormone preparation[1] was administered in divided doses over a 48-hour fasting period to male CBA mice. The total nitrogen, water and fat content of liver, lymphoid tissue, and carcass were determined at autopsy. A total dose of 200 micrograms decreased the loss of nitrogen from liver and carcass, but apparently did not affect lymphoid tissue. A significant increase in total liver wet weight was produced. This appeared due to fatty infiltration, accompanied by decreases in percentage of hepatic water and nitrogen. The increase in liver mass, however, prevented the total liver nitrogen from reaching as low a level as that observed in the controls. Carcass fat stores seemed depleted to a greater extent in the growth hormone-treated animals than in the controls, suggesting that the accumulation of liver fat was due to accelerated fat metabolism. Higher doses of growth hormone exaggerated the above effects, and, in addition, accelerated the fasting lymphoid tissue. Adrenalectomy prevented these effects on lymphoid tissue. These results indicate that the sparing of protein by growth hormone administration during fasting was associated with accelerated fat metabolism.

27. Unpredictable Effects of Growth Hormone Preparations on Nitrogen Storage.

Paul Bartlett and Oliver H. Gaebler. From the Department of Biochemistry, Research Institute, Henry Ford Hospital, Detroit, Mich.

During the past two years various anterior pituitary growth preparations which were assayed for us by the method employing plateaued rats, by the epiphyseal disc method, or by both methods, have been tested for their effect on nitrogen storage in the dog. The effect on nitrogen storage by our procedure (*J. Exp. Med.* **57**: 349, 1933; *J. Biol. Chem.* **121**: 497, 1937; **129**: 559, 1939) was not predictable from the other assays. Eventually the effects on weight, water intake, urine volume, and nitrogen balance described in the earlier papers cited above, in which "Antuitrin G" was used, were all duplicated qualitatively with preparations made according to the recent procedure of Fishman, Wilhelmi, and Russell (*Science*. **106**: 402, 1947). Meanwhile every conceivable result had been obtained with other very active preparations. A summary of our findings seems indicated for two reasons: 1) the effect of growth preparations on nitrogen or phosphate output in the urine of dogs on constant diets would be a rapid method of assay if it were consistent; 2) growth preparations assayed by other methods are apt to be used clinically on the assumption that they will promote nitrogen storage.

Our principal findings are as follows: 1. In the early experiments the amount of nitrogen stored after subcutaneous injection of 40 cc. of the same lot of Antuitrin G varied from 7.56 to 16.28 grams in 6 experiments. 2. The best storage observed in our present experiments equals the average in the former experiments only if it is calculated on a per

* This investigation was aided by a grant from the Josiah Macy, Jr. Foundation.

[1] Obtained through the courtesy of Dr. A. Wilhelmi.

kilo basis, as suggested by Kochakian (Vitamins and Hormones, IV, p. 259) in connection with anabolic effects of steroids on protein metabolism. 3. Nitrogen storage has been observed without gain in weight, as in experiments of Gordan, Bennett, Li, and Evans (*Proc. Soc. Exper. Biol. & Med.* **65**: 317, 1947) in which a dietary factor, methionine, was shown to affect the action of growth hormone. Our diet was, however, high in casein. 4. Fall in urinary phosphate has been seen following injection of pituitary preparations which produced neither gain in weight nor storage of nitrogen. 5. Attempts to show that synergism of contaminating hormones might be required for production of nitrogen storage by growth hormone preparations which were not particularly potent in this respect were negative, hence inconclusive. 6. Attempts to improve the effect of such preparations by increasing a dietary factor, riboflavin, were also negative. The basis of these attempts was that diets used in early studies in this laboratory contained much beef heart, which is rich in riboflavin.

28. Studies in Growth: I. The Effects of Androgen in Gigantism and Acromegaly.

Laurence W. Kinsell, George D. Michaels, Choh Hao Li, William E. Larsen. Division of Medicine, University of California Medical School; Institute of Experimental Biology, University of California; and Department of Medicine, United States Naval Hospital: San Francisco, Berkeley, and Oakland, California.

The apparent inhibition of pituitary growth factor production in acromegalics by the administration of estrogen has previously been reported. In this previous work it was postulated that the serum inorganic phosphorus level, other phosphate regulating factors being normal, served as an index of pituitary growth hormone production and/or activity: and it was noted that the inorganic serum phosphorus fell to normal levels during estorgen administration.

In the work here to be reported it was postulated that androgen (testosterone) would also serve as an inhibitor of pituitary growth hormone and that this material, therefore, would serve as a therapeutic agent in the control of gigantism and acromegaly in the male.

Metabolic studies over a total period of 60 days were performed on a 20-year-old male patient with active gigantism and on a 28-year-old male patient with active acromegaly. In brief, the pertinent findings were as follows:

1. Both patients, prior to treatment, had high serum phosphorus values and the patient with gigantism also had significant elevation of the serum alkaline phosphatase.

2. During the administration of testosterone propionate the serum phosphorus values on both patients dropped to normal levels. The phosphatase level was unaffected.

3. Following the cessation of testosterone administration, the serum phosphorus levels again became abnormal.

4. Visual field changes in the patient with gigantism showed no progression during testosterone administration as compared to steady progression prior to such medication. The acromegalic showed no encroachment on the visual fields.

5. Methyltestosterone does not appear to exert the desirable effects produced by testosterone propionate in these patients.

6. Balance data dealing with calcium, phosphorus, nitrogen, amino acid, and creatine metabolism will be presented and discussed as time will permit; as will quantitative growth hormone studies now in progress.

29. The Effect of Iodine Injections on Energy Metabolism and Plasma Protein-Bound Iodine of Rats.

S. B. Barker and H. J. Lipner. From the Department of Physiology, State University of Iowa, Iowa City.

A study is being made of the thyroid regulation of the metabolism of the tissues of the body through the formation and release of protein-bound iodine (PI). As part of this investigation, the effect of subcutaneously-injected elemental iodine were studied on the energy metabolism and plasma PI values of rats. The iodine was dissolved in propylene glycol and administered at one of two dose levels: 4 or 16 mg. I_2/Kg. body wt./day.

The lower dose produced no change in basal metabolism of either normal or thiouracil-treated animals during a course of four weeks' injections, despite a marked rise in the plasma PI values to 50–110 mg. per cent.

The higher dose resulted in a prompt rise in the basal metabolism of thyroidectomized animals, and a slower, less definite effect on those treated with thiouracil. Normal animals showed no response over four weeks and only a slight rise in metabolism in the fifth week. The plasma PI levels were 150–300 mg. per cent.

30. The Effect of Pituitary and Nonpituitary Gland Factors on the Formation of Intracellular Colloid Droplets in the Thyroid Gland in Vivo and Vitro.

Samuel Dvoskin, (Ayerst, McKenna and Harrison Fellow). Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York.

The administration of small doses of pituitary gland extract of the hypophysectomized rat was promptly followed by the formation of numerous intracellular colloid droplets in the thyroid epithelium. There was a direct relation between dosage and the number of droplets. Cold-exposure or thiouracil-feeding stimulated droplet formation in the thyroids of normal rats. Hypophysectomy and thyroxine administration were found to prevent or inhibit droplet formation during cold-exposure or thiouracil-feeding.

However, toxic dosages of histamine, pilocarpine or typhoid vaccine also stimulated droplet formation in the hypophysectomized rat. Human sera from normal individuals or from patients with thyroid disease were also very toxic, and induced droplet formation. The number of droplets which formed was unrelated to the clinical status of the donor. Rat sera were nontoxic and positive responses were obtained only with sera from thyroidectomized or thiouracil-fed rats.

Pituitary gland extract also stimulated formation of droplets in chick thyroids in vitro. However, droplets also formed in vitro without added pituitary extract in the central ischemic portions of the thyroid tissue. This response paralleled autolysis, and was inhibited by cooling the tissue, or by incubation with elemental iodine.

31. Inactivation of the Exophthalmic, Thyrotropic and Ketogenic Principles of Anterior Pituitary Extract by Iodination.

William McK. Jefferies. Massachusetts General Hospital, Boston, Mass.

A crude preparation of beef anterior pituitary extract was evaluated for its exophthalmic, thyroid-stimulating and fat-depositing effects before and after iodination.

By the use of a specially designed instrument and suitable technic it was observed that a quantity of anterior pituitary extract containing as little as 5 Junkmann-Schoeller units of thyrotropic hormone produced a significant proptosis of the eyes and deposition of fat in the liver and muscles of guinea pigs within 48 hours.

Male guinea pigs weighing approximately 200 grams received by subcutaneous injec-

tion anterior pituitary extract containing the equivalent of 10 Junkmann-Schoeller units of TSH or iodinated TSH daily for two days. The intercorneal distances and body weights of each animal were determined daily for six days prior to as well as during the period of treatment. The animals were sacrificed 24 hours after the last injection of the extract.

It was observed that untreated extract in the dose administered produced a proptosis of 1.6 to 2.7 millimeters during the period of treatment, and such animals showed pronounced fatty infiltration of the livers and muscles demonstrable by histological technic. Anterior pituitary extract treated with dilute Lugol's solution in vitro was found to have no thyrotropic activity as measured by chick thyroid weights and to produce no exophthalmos nor fatty infiltration of the livers or muscles in guinea pigs.

The subcutaneous administration of potassium iodide or dilute Lugol's solution with untreated anterior pituitary extract containing active TSH did not prevent the development of exophthalmos nor the deposition of fat in the tissues.

32. New Methods of Antagonizing Hyperthyroidism.

Robert H. Williams, Rene F. Tagnon, Herbert Jaffé, Beverly T. Towery, and Walter F. Rogers, Jr. Thorndike Memorial Laboratory, Boston City Hospital.

The antithyroxin capacity of butyl-, benzyl- and p-nitrophenyl-ethyl-ether of diiodotyrosine, each of which has been studied by Woolley, was estimated in rats and tadpoles (Xenopus laevis). None of the compounds lowered the basal metabolic rate of normal rats nor the QO_2 of their liver, but each slightly antagonized the calorigenic action of thyroxin. The butyl derivative was more effective than were the others, but it was also more toxic. Each of these compounds was mildly antagonistic to the metamorphotic and toxic effect of thyroxin.

Parenteral and oral administration of p-nitrophenylethyl ether to one thyrotoxic subject was without significant effect.

Iodinated thiouracil, synthesized and investigated in animals by Gassner and colleagues, has been studied in 18 thyrotoxic patients. Using total daily doses of 100 to 150 mg. of 5-iodo-2-thiouracil there was a favorable response in 7 to 14 days in all cases. However, in 6 patients the remission was not quite complete within this interval. No toxic effects were produced. Although a response simulating that obtained with thiouracil has been anticipated, the improvement in most of the patients was much more rapid. In this respect the results resemble those obtained following iodide therapy. Thus far, however, they have been more favorable.

The capacity of the thyroid gland to concentrate thiouracil and radioactive iodine following the administration of iodothiouracil is also to be reported.

33. The Use of Radioactive Iodine (I^{131}) in the Study of Normal and Disordered Thyroid Function in Man.

Sidney C. Werner and Edith Quimby. From the Departments of Medicine and of Radiological Research, Columbia University, College of Physicians and Surgeons, and the Presbyterian Hospital, New York.

The results of approximately 250 traced doses of radioactive iodine (I^{131}) in patients with varying clinical situations have been analyzed. The twenty-four hour uptake by the thyroid gland and, in most instances, the excretion in the urine over this period have been observed. A 40 to 100 microcurie dosage has been used. Various disorders in which thyroid function might be expected to be disturbed, such as those of the pituitary,

thyroid and adrenal glands have been studied. Also, the effects of antithyroid drugs such as iodine and propylthiouracil, of thyroid administration and of preceding therapy with radioactive idoine on the uptake of the radioactive isomer have been summarized. Finally, the low basal metabolic rates of anorexia nervosa and of inanition have been studied. A group of individuals without expected thyroid derangement have been observed as controls.

The correlation between basal metabolic rate fasting cholesterol and radioiodine uptake is good except that there is a group of patients in which there is a disagreement between the results of the first two, and of the last, determinations. This discrepancy reappears in the anorexia nervosa patients and after radioiodine therapy for toxic goitre. These results are of interest from the point of view of mechanisms involved.

34. The Effect of Thyroid Stimulating Hormone on the Function of Human Normal and Malignant Thyroid Tissue.

J. B. Trunnell (by invitation), R. W. Rawson, L. D. Marinelli (by invitation), Ruth Hill (by invitation). Sloan-Kettering Institute for Cancer Research, New York City.

Thyroid-stimulating hormone (T.S.H.) has been administered to seven patients with various types of cancer of the thyroid gland. The effect of T.S.H. on the function of both the normal glandular tissue and the malignant derivatives was studied with the aid of radioactive iodine. The following changes were observed:

1) An increased rate of loss of previously collected iodine.
2) An increased avidity for iodine.
3) A refractory period during which practically no iodine was collected.

There appeared to be some difference in the effect of T.S.H. in euthyroid and in hypothyroid individuals.

35. The Relation Between Infant Birthweight and Subsequent Development of Maternal Diabetes Mellitus.

Joseph P. Kriss and Palmer H. Futcher (introduced by Cyril M. MacBryde). Department of Internal Medicine, Washington University School of Medicine and Barnes Hospital, St. Louis, Mo.

Analysis was made of birthweight data on infants born to 100 women destined later to develop diabetes and on infants born to 100 nondiabetic (control) women of comparable age and parity. In the 2 groups there were 144 infants weighing 10 pounds or more; 77.1% of the 144 were born to prediabetic women. Of 52 infants weighing 12 pounds or more, 90.3% were born to prediabetic women. Single and repeated birth of abnormally large infants occurred far more commonly in the prediabetic mothers than in the control mothers. As reported by H. C. Miller, the average birthweight of the infants born to the prediabetic mothers was significantly greater than that of infants born to control mothers. The period between the birth of the first abnormally large infant and the development of clinical diabetes in the mother averaged about 24 years, with a range from 1 to 46 years.

According to Spiefelman and Marks, it may be predicted that 4% of women of childbearing age will develop diabetes. The data on our small group of subjects indicate that diabetes developed in over 50% of women giving birth to one baby weighing over 13 pounds or to 3 babies weighing more than 10 pounds.

2:00 P.M. ***Red Lacquer Room***

36. ABSORPTION AND EXCRETION OF CHORIONIC GONADOTROPIN WHEN ADMINISTERED INTRAMUSCULARLY TO WOMEN. A COMPARISON OF AQUEOUS, OIL EMULSION AND OIL AND WAX PREPARATIONS.

J. T. Bradbury and Willis E. Brown. Department of Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, Iowa.

This study was designed to investigate the relative rate of absorption of chorionic gonadotropin from various injection media. A single dose of 10,000 I.U.was given intramuscularly to normal women and the rate of excretion in the urine was determined at 12-hour intervals up to 120 hours. The aqueous solution was Antuitrin-S (Parke, Davis and Company) and was made into an oil emulsion with Emulgen (Lakeside). The wax and oil preparation was A.P.L. (Ayerst, McKenna, Harrison).

The urinary assays were made by injecting the test rats with urine in volumes equivalent to 1, 3, or 6 minutes output. Positive assays were those in which definite corpora lutea were present at 96 hours.

Absorption from the aqueous solution was very rapid since urine volumes equivalent to a 3-minute output contained at least one rat unit by the twelfth hour after injection. This rate of urinary excretion was not reached until 12 to 24 hours after administering the hormone in an oil emulsion or in oil and wax. From 12 to 48 hours after injection in an aqueous media the excretion rate was such that a one-minute urine output occasionally contained 1 rat unit but the excretion rate after injection in oily media did not reach this value. From 48 to 72 hours the excretion rate was rather uniform, a 3-minute urine volume containing about 1 rat unit, irrespective of which media had been injected. From 72 to 120 hours the amount of gonadotropin in the urine was consistently greater after being injected in an aqueous solution.

These observations would suggest that the absorption from oily media may be too slow to result in optimum blood levels. It may be that the rate of elimination of this protein hormone through the human kidney is so slow that there is no real advantage in trying to delay its absorption at the site of injection.

37. THE RENAL CLEARANCE OF CHORIONIC GONADOTROPIC HORMONE IN PREGNANCY AND IN NEOPLASM OF THE TESTIS.

C. F. Gastineau (by invitation), A. Albert and L. M. Randall (by invitation). Mayo Clinic, Rochester, Minnesota.

The concentrations of chorionic gonadotropin in the serum and the urine of human subjects were determined (by Albert *see* abstract No. 80 in this program), and the renal clearances were calculated. A total of 20 such clearances were made on 5 normal pregnant women and 1 on a patient with chorionepithelioma of the testicle. The average clearance in pregnancy was found to be 0.38 ± 0.14* ml./min.; that for the first trimester, 0.44 ± 0.10 ml./min.; that for the second trimester, 0.37 ± 0.17 ml./min.; and that for the third trimester, 0.36 ± 0.10 ml./min. Thus, the clearance was relatively constant during all stages of pregnancy although the serum and urinary concentrations exhibited as much as a twentyfold variation.

The concentrations of hormone in the patient with testicular tumor were: urine, 9.6 I.U./ml.; serum, 28 I.U./ml.; and neoplastic tissue, 220 I.U./Gm. The clearance was 0.23 ml./min., a value in the lower limit of those found in pregnancy.

These studies show that the normal human kidney clears chorionic gonadotropin in a relatively constant rate. Furthermore, results indicate that with normal renal function,

* Standard deviation.

changes in urinary excretion are reflections of changes in serum level and therefore probably depend mainly on changes in rate of production of the hormone.

38. The Metabolic Response to Chorionic Gonadotropin Stimulation in Young Men.

Kathryn Knowlton and Allan T. Kenyon. From the Department of Medicine, University of Chicago.

Testosterone is known to be an anabolic agent in man. Chorionic gonadotropin given to a responsive boy has produced like effects, presumably through increased androgen production by the testis. Such stimulation has not yet been studied, however, in the mature male. The following findings indicate its consequences.

One normal man received 500 to 3000 I.U. chorionic gonadotropin daily for 6 days, another 500 to 1500 units daily for 12 days. Nitrogen retentions were 60 and 22 mg./Kg./day respectively. Creatine and inorganic phosphorus were similarly retained. This anabolic response may well be due to an output of testicular androgens greater than that obtained in the state of endocrine balance before gonadotropin administration. The normal mature testis is thus probably sensitive to an additional stimulus of similar nature to that already acting on it.

The effects of chorionic gonadotropin in a eunuchoid individual with inactive testes were irregular and minimal. This may be interpreted as representing stimulation of residual interstitial cells or as indicating a response of other structures or processes. Such response, however, was much less than that induced by 5 mg. testosterone propionate daily for 6 days in the same patient.

39. Blood Gonadotropin Studies During Pregnancy in Relation to the Fetal Sex.

H. E. Nieburgs and Robert B. Greenblatt, School of Medicine, University of Georgia, Augusta, Ga.

In a series of 253 pregnant women specific estrogenic and androgenic smears occurred in 37.5 per cent. Of these, 88.1 per cent were delivered of infants whose sex corresponded to the specific smear. A further study on 89 pregnant women yielded confirmation on these findings.

In a group of 64 women blood gonadotropin assays carried out between the seventeenth and thirty-second week of pregnancy revealed a close relationship of the FSH:LH ratio of the sex of the infant.

The test is performed by the injection of 1 cc. of serum into immature female white mice both as whole serum and also in three dilutions over three days. At autopsy 48 hours after the last injection, FSH is estimated on the basis of ovarian stimulation, uterine weight increase and vaginal cytology, while the level of LH is established by the presence of hemorrhagic follicles or corpora lutea. A predominantly high level of FSH was associated with a female infant while an equal level of LH and FSH was always followed by a male infant. When 3 cc. of the same serum was injected into immature female rats the same ratio of FSH to LH was obtained. Serum with a level of LH equal to FSH injected into immature male rats in the dose of 3 cc. produced descent of the testes and an increase in weight of the prostate, while serum with a predominantly high FSH titer failed to produce these results.

In those cases where a specific vaginal smear was obtained, the androgenic type was associated with a high LH titer while the estrogenic smear occurred in the presence of an increased FSH level. Observations on a further group of 30 pregnant women are presented. Lantern slides.

40. On the Principal Estrogenic Constituents of the Urine of the Stallion.
Louis Levin. Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, N. Y.

Continued investigation of the estrogenic constitutents of stallion urine has confirmed and extended the previous finding (Levin, *J. Biol. Chem.* **158**: 725, 1945) that such urines, when fresh, contain large quantities of estrogenic substances (average 54,000 R.U./liter) and that a major proportion of the estrogenic activity is attributable to the presence of α-estradiol. Every specimen of stallion urine thus far examined has contained large quantities of estradiol, 42 to 90 per cent (average 56 per cent) of the total estrogenic activity being due to this substance. Stallion urine is therefore the richest known natural source of α-estradiol.

The presence of α-estradiol has been confirmed by distribution coefficients, separation with ketonic reagents, determination of ratio of rat unit to mouse unit, comparison of biological activity to photo-fluorescence in 70% H_2SO_4, and in several cases by isolation of the estradiol as the di-α-naphthoate.

That the presence of α-estradiol is not due to bacterial reduction of preformed estrone has been established by allowing a number of specimens to age, exposed to air, for varying periods. In no case did the estradiol content increase. Conversely, in several specimens, the estradiol content diminished with age with a proportionate increase in the estrone content, indicating an oxidative conversion of preformed estradiol to estrone. This may explain the failure of some investigators to find estradiol in stallion urine.

Control studies of the estrogenic constituents of urine of pregnant women and of pregnant mares have yielded estrone/estradiol ratios similar to those obtained in other laboratories, proving that the high titers of estradiol found in stallion urine are not due to inadvertent conversion of estrone to estradiol during the hydrolysis, separation and purification.

41. Mechanism of Inactivation of α-Estradiol by Rat Liver "In Vitro."
R. H. DeMeio (by invitation), A. E. Rakoff, A. Cantarow and J. E. Paschkis. Jefferson Medical College, Philadelphia, Pa.

The inactivation was studied by incubating either male-rat liver slices (100 mg.) or homogenate (10 per cent) in 5 ml. of Krebs solution, phosphate buffer pH 7.4, without glucose for two hours at 37.5° C. Usually 1γ of α-estradiol per millileter was used. The amount of biologically active material present at the end was determined by bioassay in mice.

Complete inactivation by liver slices was observed whether the gas phase was oxygen or air. In nitrogen or with boiled slices no inactivation took place.

The 10 per cent homogenate prepared with the Waring blender or by crushing the frozen liver in a mortar to a fine powder produced from 40 to 70 per cent inactivation. No change in the behavior of the homogenate was observed when the Fuhrman and Crismon "intracellular medium" was substituted for the Krebs solution or when boiled liver extract or glucose was added to the medium.

The inactivation by liver slices was inhibited by capryl alcohol, but not by cyanide, azide, thiourea, malonate, fluoride or monoiodoacetate.

The addition of methylene blue to slices incubated in nitrogen brought about a 40 to 70 per cent inactivation.

Nicotinamide and DPN added to the homogenate increased the inactivation by approximately 50 per cent.

Our results indicate that no appreciable inactivation is due to conjugation of the estradiol.

As a consequence of our findings we may conclude that the system cytochrome-cytochrome oxidase does not participate, or if it does, only to a minor extent; and that the inactivation is probably produced by a dehydrogenating system or systems.

42. Cozymase in the Hepatic Inactivation of α-Estradiol.

Richard L. Coppedge, Albert Segaloff, Herbert Sarett and Aaron Altschul. From Tulane University and the Alton Ochsner Medical Foundation, New Orleans, La.

In an effort to isolate the enzymes responsible for the hepatic inactivation of α-estradiol, varying amounts of liver tissue were incubated with varying amounts of α-estradiol and assayed by the uterine weight increases of immature female rats. Comparison of tissue slices and tissue mince from the same rat liver in their relative abilities to inactivate α-estradiol demonstrated a decided reduction in the capacity of the mince for inactivation.

This suggested that a component of the inactivating system was unstable in a broken-cell preparation and might be enzyme-labile. When supernatant material from rat-liver slices boiled in physiological saline was added to liver mince, the ability of the mince to inactivate α-estradiol was increased to that of slices from the same liver. The heat-stable component in the supernatant material was suspected to be cozymase (diphosphopyridine nucleotide) since cozymase is rapidly inactivated in tissue mince.

Mince prepared in a solution containing nicotinamide, which protects cozymase, possessed an estrogen-inactivating ability equal to that of slices from the same liver.

Addition of cozymase to mince simultaneously with α-estradiol also increased its estrogen-inactivating capacity to that of slices. However, when cozymase was allowed to stand with mince for one-half hour prior to addition of the estrogen, this increased inactivating capacity was lost. Addition of nicotinamide after liver had been minced, but prior to addition of cozymase, prevented this loss.

The degree of inactivation is found to be, within limits tested, a function of the amount of cozymase added.

43. Interference with Estrogen-Induced Growth in the Female Genital Tract by Folic Acid Antagonist.

Roy Hertz. National Cancer Institute, Bethesda, Maryland.

Earlier observations have indicated that both the monkey and chick require folic acid for optimum tissue-growth response to estrogen. Moreover, in the chick a direct quantitative relationship between the dietary intake of folic acid and the degree of estrogen response has been demonstrated.

In accordance with the principle of competitive metabolites, a number of folic acid antagonists have been synthesized. The potency of a series of these folic acid antagonists as inhibitors of the tissue-growth response to estrogens will be described.

The data indicate that it is possible to interfere with the biological activity of a hormone by the use of an anti-vitamin. The potential clinical and experimental usefulness of this mechanism will be discussed.

44. The Relation of Folic Acid to the Action of Estrogens.

Irene T. Kline and Ralph I. Dorfman (with the technical assistance of Patricia McCreery). From the Departments of Biochemistry and Medicine, Western Reserve University School of Medicine and Lakeside Hospital, Cleveland 6, Ohio.

Female chicks were placed on a complete synthetic diet with adequate supplements of all known vitamins but were limited in their food intake (group A) so that their final body weights were significantly lower than those which received an adequate amount of food (group B). When chicks in groups A and B were treated with a total of 1.25 mg.

of stilbestrol over a period of five days no significant difference in oviduct weight could be demonstrated. Although animals in group B were stunted, a full oviduct response to stilbestrol was elicited.

When folic acid was removed selectively from the diet the oviduct showed almost no response to either stilbestrol (confirmation of Hertz, R: *Endocrinology* **37**: 1, 1945) or estrone. Statistically significant decreased oviduct responses to stilbestrol were observed when the following deficient diets were used: pyridoxine; pyridoxine and paraminobenzoic acid; riboflavin; riboflavin and paraminobenzoic acid; or nicotinic acid. No significant change in oviduct responses to stilbestrol were observed when the following deficient diets were used: inositol; paraminobenzoic acid; or a α-tocopherol. Significant decreases in body weight were observed in all cases of deficiencies mentioned.

45. Fluorescent Phenomena of the Vulva Associated with Sex Hormone Metabolism.

M. Sydney Margolese. Winnipeg, Canada.

Irradiation of the vulva with ultraviolet light results in fluorescence exhibiting various colors. This phenomenon was studied in 200 individuals, and over 2500 color pictures were recorded, covering normal and abnormal menstrual cycles, infancy, childhood, adolescence, pregnancy, the menopause, and the postmenopause.

The study shows that there is a relationship between the colors observed, and the ovarian hormone levels. Hence visual study of vulvar fluorescence offers a simple and practical method of estimating the ovarian hormones qualitatively.

The method may be applied to the following:

1. Diagnosis of disorders of the menstrual cycle by the estimation of deviations in estrogen and progesterone levels.
2. Determination of the time of appearance of the ovarian hormones during childhood and adolescence.
3. Diagnosis and evaluation of treatment of the climacteric.
4. The differentiation of endocrine and nonendocrine vaginal bleeding.
5. Diagnosis of threatened abortion prior to the advent of other signs or symptoms.
6. A guide in the treatment of threatened abortion of endocrine origin.

46. Testicular Deficiency: A Clinical and Pathological Study.

R. Palmer Howard, Ronald C. Sniffen (by invitation), and Fred A. Simmons. From the Departments of Medicine, Pathology and Surgery and the Biological Laboratory, the Massachusetts General Hospital, Boston, Mass.

In the study of 142 men, complaining of sterility or suffering from various endocrine disorders, histological examinations of testes have been correlated with clinical findings and urinary gonadotropin (FSH) and 17-ketosteroid excretions. Cases are divided into three large groups. (A) *Hypogonadotropic conditions.* If gonadotropic stimulation has never occurred the testes resemble prepuberal glands. When gonadotropic suppression has occurred after testicular maturity the histological features are fat-laden Sertoli cells, hypospermatogenesis, thickening of the tubular basement membrane, appearance of peritubular elastic fibrils and absence of normal Leydig cells. (B) *Primary testicular deficiency without endocrine manifestations.* Various disturbances in spermatogenesis are encountered but Sertoli cells, basement membrane and Leydig cells are usually normal. (C) *Primary testicular deficiency with elevated gonadotropins.* Included are (i) absence of testes; (ii) histologically normal gonads in the climacteric; and (iii) tubular sclerosis involving the Sertoli cells and the germinal epithelium, associated with clumped atypical Leydig cells, and often accompanied by gynecomastia.

It is proposed that gonadotropin (presumably follicle-stimulating hormone) stimu-

lates the Sertoli cells which secrete a hormone that promotes spermatogenesis, inhibits FSH production, and possibly influences other gonadotropins which act on the Leydig cells.

47. A Comparison of the Effect of Various Androgens on the Temporal Muscle and Organs of the Castrated Male Guinea Pig.

Charles D. Kochakian and Jane Harrison Humm. School of Medicine and Dentistry, University of Rochester, Rochester, New York.

Castration of immature male guinea pigs (250 ± Gm.) resulted in no change in the weight of the kidneys or the adrenals but did produce the expected atrophy of the seminal vesicles and prostates and the temporal muscle. The ability of androgens implanted subcutaneously as pellets for 30 days to increase the weight of the temporal muscle was: androstanol-17α, one-3; 17-methylandrostanol-17α, one-3;>testosterone propionate; testosterone;>androstanediol-3α, 17α, 17-methylandrostanediol-3α, 17α; 17-methyltestosterone. The 17-methyl steroids, excepting the case of 17-methyltestosterone, had approximately the same activity as their non-methylated compounds.

The myotrophic androgenic ratio (increase in temporal muscle weight divided by the increase in seminal vesicle and prostate weight) was: androstanediol-3α, 17α (0.49); 17-methylandrostanediol-3α, 17α (0.45); normal (0.31); 17-methylandrostanol-17α, one-3 (0.26); androstanol-17α, one-3 (0.23); testosterone propionate (0.17); testosterone (0.17); 17-methyltestosterone (0.14).

Analyses of the muscles for water, nitrogen and nonprotein nitrogen indicated that the increase in muscle mass under steroid stimulation had the same composition as the muscle of normal guinea pigs.

The weights of the adrenals and the kidneys were not significantly altered by any of the androgens.

The body weights were significantly increased by the various androgens.

Papers Read by Title

48. The Use of Whole Adrenal Cortical Extract in Experimental Infections.

Erwin P. Vollmer, James D. Gillmore, Leo Cravitz and J. E. Samsell. From the Bacteriology Facility, Naval Medical Research Institute, Bethesda, Md.

Because of the numerous indications that adrenal cortical function may be a factor in resistance to infection, information has been sought about the outcome of experimental infections in intact animals treated with adrenal cortical extract (ACE). Extended survival time in ACE-treated guinea pigs infected with *Clostridum welchii* (previously reported by Kepl *et al.*) was confirmed, 16 treated animals living on the average twice as long (60 hours) as 16 saline-treated controls. Survival time in small groups of animals may, however, be an undependable criterion. Significant differences in survival were obtained in two experiments with Type I *Pneumococcus* in Swiss mice (ACE-treated 22/100 and 16/40, as against saline-treated 0/100 and 0/40, respectively), when light inocula were injected intraperitoneally and two or more doses of ACE were given. Similarly designed experiments sometimes gave insignificant results, as did also several experiments involving subcutaneous inoculations of pneumococcus or the intraperitoneal inoculation of group A, B-hemolytic streptococcus. Elicitation of an anamnestic response in immunized mice, by injection of ACE 2 to 3 hours before challenge had no effect on the outcome of pneumococcal infection. It is concluded that despite occasional evidence that ACE administered during infection may benefit intact animals, clear understanding of the role of the adrenal cortex in resistance to disease may require further extensive exploration.

49. The Work Performance of Adrenalectomized Rats Given Continuous Intravenous Infusions of Glucose.

Dwight J. Ingle and James E. Nezamis. Research Laboratories, The Upjohn Company, Kalamazoo, Michigan.

The work performance of the adrenalectomized-nephrectomized rat is a sensitive index of the biologic activity of adrenal cortical extracts and C_{11} oxygenated steroids (Ingle, D. J.: *Endocrinology* **24**: 191, 1944). Loss of muscular responsiveness is associated with hypoglycemia. The relative potency of cortical extracts and steroids parallels very closely their activity in the liver-glycogen deposition test (Pabst, M. L.; Sheppard R. and Kuizenga M. H.: *Endocrinology* **41**: 55, 1947). It was previously shown (Ingle, D. J., and Lukens, F. D. W.: *Endocrinology* **29**: 443, 1941) that a single injection of glucose will temporarily restore muscular responsiveness in the "fatigued" adrenalectomized rat.

In the present studies different glucose loads were given by continuous intravenous infusion to adrenalectomized-nephrectomized rats during twenty-four hours of muscle stimulation. The adrenally insufficient rats tolerated greater amounts of glucose than did nonadrenalectomized rats or treated rats, but muscular performance was not improved by glucose except in the presence of the cortical hormone. Although exhaustion of available carbohydrate may be one factor limiting the work performance of the adrenally insufficient rat, the prevention of hypoglycemia does not sustain the responsiveness of of stimulated muscle in these animals.

50. Sublingual Administration of Desoxycorticosterone Acetate in the Treatment of Addison's Disease.

Evelyn Anderson, Lawrence W. Kinsell, Troy C. Daniels and Edward Henderson. The Division of Medicine, University of California Medical School; The College of Pharmacy, University of California; and the Division of Clinical Research, Schering Corporation.

For the past 8 years the patients with Addison's disease at the Medical Clinic of the University of California Hospital have been treated routinely with desoxycorticosterone acetate (DCA) sublingually. From 1940 through 1946 the method consisted of giving a solution of DCA in drops under the tongue. The solvent used for the DCA was a mixture of propylene glycol and absolute alcohol. It was found that this method of treatment controls the adrenal insufficiency satisfactorily, provided meticulous care is taken in maintaining the DCA in solution and in administering the solution in frequent small doses so as to permit absorption in the mouth and prevent the swallowing of the material. The sublingual method of administration of DCA has been considerably improved by the use of tablets of a polyethylene glycol wax in which the DCA is dissolved. In the past year 14 of our patients with Addison's disease have been treated with such tablets. The range of dosage has been from 2 to 8 mg. of DCA per day; 8 of these patients have been maintained on 4 mg. per day. A comparison of this with other methods of administration of DCA is discussed.

51. Excretion of Adrenal Metabolites Following the Administration of Adrenocorticotropic Hormone to Normal Human Subjects.

Eleanor H. Venning, V. E. Kazmin (by invitation), Miriam Ripstein (by invitation), H. T. McAlpine (by invitation) and M. M. Hoffman. McGill University Clinic, Royal Victoria Hospital, Montreal.

Comparative studies have been carried out on the excretion of adrenal metabolites following the administration of large quantities of adrenocorticotropic hormone (240 to

420 mg., equivalent to 120 to 210 mg. of the Armour standard preparation (LA-1-A). Substances measured were the glycogenic corticoids by means of the bioassay method of Venning, Kazmin and Bell, the 11-oxysteroids by the method of Talbot *et al.* and the 17-ketosteroids by the method of Holtorff and Koch. The benzene-soluble, the water-soluble nonketonic and the water-soluble ketonic fractions were examined. These studies indicate that there is a rapid rise demonstrable in 12 hours in the glycogenic corticoids and 11-oxysteroids. The glycogenic corticoid values rose from normal levels to as high as 480 glycogenic units in the first 24 hours. In the second 24-hour period after the cessation of injections the values had all returned to normal levels.

52. The Effect of 11-Dehydrocorticosterone on Fecal Fat Excretion.

Grace E. Bergner (by invitation), Roger A. Lewis, Frances W. Stout (by invitation), George W. Thorn, and Kendall Emerson, Jr. Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

A group of nine adrenalectomized rats maintained with pellets of desoxycorticosterone acetate and tube-fed a constant diet excreted a quantity of stool fat and nitrogen comparable to that observed for a control group of normal animals. The stool analyses were performed on composite specimens obtained from all animals in each group. In the group of adrenalectomized animals a decrease of 50 per cent in total fecal fat excretion was observed during a period on Compound A therapy, i.e. 5 mg. in sesame oil administered twice daily intramuscularly for three days. The fecal fat excretion fell only 14 per cent in the control group. The fecal nitrogen excretion remained essentially unchanged in both groups. The biological activity of the Compound A was indicated by a 65 per cent increase in the urinary nitrogen excretion in the adrenalectomized group and a 21 per cent increase in the control group.

In two patients with Addison's disease without appreciable steatorrhea the daily administration of 60 mg. of Compound A in oil in divided doses was associated with a decrease in fecal excretion of fat of 45 and 52 per cent respectively. The diet was constant throughout these studies. A third patient with Addison's disease associated with appreciable steatorrhea had a 75 per cent decrease in fecal fat excretion on a similar dosage. In a fourth patient fecal fat/nitrogen ratio was followed with and without the administration of 60 mg. of the water-soluble hemisuccinate of 11-dehydrocorticosterone. The fecal fat/nitrogen ratio fell 56 per cent. Thus the changes observed in patients with Addison's disease are comparable to the effects shown in adrenalectomized rats.

Additional studies of this type have been precluded by the unavailability of synthetic 11-dehydrocorticosterone.

53. An Estimation of the Quantity of 11-17-Oxysteroid Excretion by the Human Adrenal Stimulated by ACTH.

A. Gorman Hills (by invitation) and George W. Thorn. Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

A fall in circulating eosinophils was previously shown to be a highly specific and sensitive index of the secretion of 11-17-oxysteroids by the adrenal cortex. The mean magnitude of the fall resulting from adrenal-stimulation by a single injection of ACTH has been found to be a function of the dose employed. A dose-response curve has been constructed for ACTH based upon multiple determinations of the change in circulating eosinophil count following the administration of varying doses of ACTH to patients without evident abnormalities of adrenal function. By comparing the response of six

patients with Addison's disease given a single injection of 12 mg. of crystalline 17-hydroxycorticosterone with the dose-response curve of normal subjects given ACTH an approximate estimation has been made that 4 mg. of purified hog ACTH administered to human subjects is followed by the secretion of a quantity of 11-17-oxysteroids equivalent to 12 mg. of Compound F. Changes in the eosinophil count have also been used as a means of estimating the quantity of 11-17-oxysteroids liberated in patients who have undergone a major operative procedure.

54. Isolation of Urinary Steroids from a Patient with Apparent Adrenal Involvement.

A. M. Miller and Ralph I. Dorfman. Departments of Biochemistry and Medicine, Western Reserve University School of Medicine and Lakeside Hospital, Cleveland 6, Ohio.

A study was made of the neutral steroids of the urine from a virilized girl. The following compounds were identified: Δ^2 or ^{3}-androsterone-17, androstanediol-3(α), 11(β)-one-17 (10 mg./liter), Δ^9-androstenol-3(α)-one-17, androsterone, 3-chloro-Δ^5-androsterone-17, etiocholanol-3(α)-one-17, dehydroisoandrosterone, pregnanediol-3(α), 20(α), allopregnanediol-3(α), 20(α), etiocholanedial-3(α), 17(α), and pregnanetriol-3(α), 17, 20 (8 mg./liter).

In addition to those compounds which have been identified a series of compounds have been isolated by chromatographic analysis of the acetates of the ketonic and nonketonic fractions respectively. From the ketonic fraction the following compounds have been isolated: compound I, m.p. 132-4° C. (not Δ^4-androstenedione-3, 17); compound II, m.p. 178–182.5° C.; compound III, m.p. 236–237.5° C.; compound IV, m.p. 169° C.; compound V, m.p. 199–200° C; and compound VI, m.p. 199–200° C. From the nonketonic fraction the following compounds have been isolated: compound VII, m.p. 144–144.5° C.; and compound VIII, m.p. 225–226° C.

55. The Effect of Adrenalectomy and Desoxycorticosterone Acetate Administration Upon the ECG Response of the Rat to Cardiac Glycosides.

Herbert S. Kupperman, Joseph G. Benton and Arthur C. DeGraff. Department of Therapeutics, New York University College of Medicine, New York, N. Y.

As part of a study of the interrelationships between the steroid hormones and cardiac glycosides, an investigation of the effect of adrenalectomy and desoxycorticosterone acetate (DCA) on the response of the rat to cardiac glycosides has been undertaken. Control ECG records were obtained prior to the induced changes in the endocrine status of the animal. Three groups of female rats weighing between 90 and 150 grams were employed. One served as control for the two experimental groups of which one received DCA and the second was adrenalectomized. All three groups of animals received intravenous injections of the rapidly acting glycoside, ouabain, as well as the slower acting preparation, digitoxin. A fourth group of control rats received intravenous injections of equal volumes of either normal saline or 25% alcohol. The effect of the glycosides as influenced by DCA administration or adrenalectomy was studied by means of ECG tracings taken at various intervals after the intravenous injection of the cardiac drugs and compared with similar tracings from the two groups of controls. Administration of the glycosides to animals receiving DCA resulted in little effect upon the ECG pattern. Furthermore, this adrenal cortical steroid rendered some animals resistant to doses of the cardiac glycosides that induced ECG effects in the control group. On the other hand, with doses that produced only miminal changes in the control animal, definite effects were observed in the adrenalectomized group. These were manifested by bundle branch

block, A-V dissociation, marked slowing of the ventricular rate and cardiac arrest in some animals. No instance of ventricular fibrillation was noted with the doses employed. The clinical implications of these findings are discussed.

56. Activation of the Adrenal Cortex in Human Subjects Following Electroconvulsive Therapy (E.C.T.) and Psychomotor Stress.

R. A. Cleghorn, and (by invitation) A. J. Goodman, B. F. Graham, M. H. Jones, and N. K. Rublee. McGill University, Montreal, Canada.

It has been demonstrated that an increase in the activity of the adrenal cortex will cause a decrease in the number of circulating lymphocytes and eosinophils, an increase in the number of neutrophils, and an increase in excretion of uric acid compared with creatinine.

Fluctuations in these indices of adrenal activity have been studied in four groups of subjects:

A. Supposedly normal persons pursuing a usual day's activities.

B. Individuals with anxiety neurosis subjected to a standard stress situation.

C. Cases receiving electroconvulsive therapy.

D. A mixed group of normal, neurotic, and endocrinopathic subjects receiving adrenocorticotropin (ACTH) in doses varying from 4.5 to 25 mg. equivalents of the Armour standard.

It is concluded 1) that group A shows evidence of fluctuations in adrenal cortical activity as reported previously by others; 2) that groups B and C display increased cortical activity corresponding to that produced by relatively small doses of ACTH in group D.

57. Effect of Adrenal Cortical Compounds on Electrolyte Metabolism of a Patient with Addison's Disease During High Sodium Chloride Intake.

Aurelia Potor, Nelson F. Young, F. Homburger and Edward C. Reifenstein, Jr. From the Department of Clinical Investigation of The Sloan-Kettering Institute, Memorial Hospital Cancer Center, New York City.

This report is the result of observations of electrolyte metabolism in a patient with Addison's disease during administration of large quantities of sodium chloride.

Urinary excretion of sodium demonstrated a notable deficit during high intake when compared with an average intake of sodium. Further comparison of urinary excretion with that in a normal individual under the same conditions demonstrated a slope ratio of 0.3 in Addison's disease and 0.98 in a normal individual.

A subsequent period of high sodium intake was followed by the administration of adrenal cortical hormone, which resulted in a notable increase in urinary sodium excretion. A duplicate period of study, using desoxycorticosterone acetate, demonstrated a less pronounced increase in urinary sodium excretion.

58. Nitrogen-Sparing (Protein-Anabolic) Action of Thyroid Hormone.

J. Rupp (by invitation) and K. E. Paschkis. Jefferson Medical College, Philadelphia.

Thyroid hormone is usually looked upon as enhancing protein catabolism. There are however indications that under certain circumstances, thyroid hormone is involved in protein anabolism as for example evidenced by the growth arrest of the cretin, and the resumption of growth following administration of thyroid.

Investigations of the conditions under which thyroid acts as a protein anabolizer are reported here.

All experiments were performed on force-fed rats in order to avoid changes in food intake occurring in ad-lib fed animals treated with thyroid. In the intact rat, large doses of thyroxin induced protein catabolism as evidenced by increased N-excretion and weight loss. Small doses (10 to 5 micrograms daily) had no effect on N-excretion or weight. In thyroidectomized rats, large doses also increased N-excretion. Following the injection of small doses there occurred a marked N-retention indicating stimulation of protein anabolism.

The results suggest that dosage of thyroid hormone and the pretreatment condition of the animal are two factors which determine the protein anabolic action of thyroxin. Other factors are under investigation.

59. Thiouracil Effect on Plasma and Liver Protein Concentrations.

James H. Leathem. Bureau of Biological Research and Department of Zoology, Rutgers University, New Brunswick, N. J.

An increase in total plasma protein due to an increase in plasma globulin concentration appears in hypothyroidism induced by thyroidectomy or by feeding antithyroid drugs. These hypothyroid states differ, in that thyroidectomy may cause liver atrophy whereas thiouracil increases liver size and total protein in the body.

Experiments on adult male rats fed thiouracil for 20 days show that a dosage of 0.1, 0.25 or 0.5 per cent of the diet increases total plasma protein concentrations to the same extent. The increase is due to plasma since globulin plasma albumin concentration is not altered. Hematocrit decrease and NPN increase occur in comparison with pair fed controls. Thiouracil with restricted food intake, and propylthiouracil, elicit similar changes. Liver weight and liver protein/100 grams body weight, increase in direct relationship to daily dosages of 0.1–0.5 per cent thiouracil fed for 20 days. Feeding 0.1 per cent thiouracil for 20 and 40 days induced similar liver changes. The goitrogen prevented liver protein and size from decreasing in rats subjected to restricted food intake.

Elevated globulin levels of hyperthyroid patients were returned to normal and maintained at normal concentrations during 15–20 months of thiouracil administration.

60. Radioiodine Uptake by the Thyroid as an Aid in Differential Diagnosis.

S. M. Seidlin, E. Oshry, I. Rossman and L. Leiter. From the Medical Division, Montefiore Hospital, New York City.

The usual methods of evaluating thyroid function are inadequate in certain clinical conditions. As is well known, the basal metabolic rate and the blood cholesterol level may undergo gross changes independent of thyroid function. The determination of the uptake of radioiodine by the thyroid gland (the fraction of the dose localized in the gland) and the urinary excretion of the isotope present a method of great usefulness in differential diagnosis when the routine methods are inapplicable. About forty patients have been studied with this method and characteristic curves have been obtained for hyperactive, normal and hypoactive thyroid glands. The method has been particularly valuable in cases where the basal metabolic rate is high due to factors other than increased thyroid activity as, for example, in cases of Parkinsonism, heart failure, or anxiety neurosis; and where the blood cholesterol determinations may be of no value as, for instance, in undernutrition or diabetes.

61. Thyroid Uptake of Radioactive Iodine in the Normal and Hypometabolic Human.

Martin Perlmutter (by invitation) and Peter H. Forsham (by invitation). Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

The rate of iodine uptake by the thyroid gland of apparently euthyroid patients was studied by the use of tracer doses (0.1 millicuri) of I^{131}. Hourly counts of radioactivity in the thyroid area were determined by the use of a Geiger-Muller counter. The rate of accumulation of iodine in thyroid tissue was calculated according to the method of Stanley and Astwood (*Endocrinology* **41**: 66, 1947).

A study of the rate of iodine uptake in patients of varying ages indicated a decreased rate of iodine uptake by the thyroid gland of elderly "normal" patients. These observations are compared with other measurements of thyroid activity such as basal metabolic rate, serum protein-bound iodine, and serum cholesterol values.

Studies of the rate of iodine uptake by the thyroid were also made in a group of patients with adrenal insufficiency, primary thyroid insufficiency, and anterior pituitary insufficiency. The accumulation gradient of iodine uptake was negative only in patients with primary thyroid insufficiency. Factors which give false low accumulation gradients are also discussed.

62. Cellular Involution in the Thyroid.

Nathan B. Friedman, Army Institute of Pathology.

By involution of the thyroid ("follicular involution") is meant that process in which follicular colloid is redeposited and follicular epithelium returns to the flattened resting state after a period of hyperplasia. This phenomenon contrasts with certain other epithelial alterations which take place under a variety of conditions and might well be termed "cellular involution." The most prominent of these changes is the formation of the eosinophilic elements commonly called Hürthle cells in lesions of the thyroid generally regarded as regressive. Recognizing that cellular involution is common to a number of diseases may aid in the understanding of the pathologic physiology of the thyroid gland.

Numerous examples of cellular involution in the thyroid have been studied during the past 5 years at the Army Institute of Pathology. The conditions in which the phenomenon was observed include Hashimoto's disease, myxedema, "exhaustion" atrophy and exophthalmic goiter. It is of particular interest that identical alterations were observed consequent to radiation therapy, treatment with thiouracil and subtotal resection. The occurrence of cellular involution can be taken as an indication that the altered glandular elements had "burned out" as a result of overstimulation. Thus the thyroid shares with certain other of the endocrine organs a tendency to enter an inactive phase subsequent to a prolonged period of excessive activity.

63. Methylthiouracil in the Treatment of Thyrotoxicosis.

Grosvenor W. Bissell, John M. Benny, Victor Totah and Florence Gilbert. Department of Medicine, University of Buffalo School of Medicine and Endocrine Clinic, E. J. Meyer Memorial Hospital, Buffalo, N. Y.

Methythiouracil has had a fairly extensive trial in Europe, where large initial and maintenance doses have been used. It has proved an effective antithyroid agent. Toxic reactions have been reported, possibly related to the high dosages employed.

We have treated 30 hyperthyroid patients with methylthiouracil, employing maximum initial divided daily doses of 250 mg. In many instances, an initial divided daily dose of 150 mg. was found effective. Most previously untreated patients gave distinct clinical evidence of improvement in 10 days, and were clinically normal in an average of 30 days. Effective daily maintenance averaged 50 mg. in divided doses.

Numerous repeated studies of peripheral blood, urine, and liver function revealed no toxic effects of the drug. Two evanescent skin rashes were observed, which disappeared without alteration of therapy. Two patients developed frank psychoses, but in both, evidence exists that there is no causal relationship to the drug. Several patients developed hypothyroidism during maintenance therapy, which responded to modifying the dosage. Two patients developed huge goiters on maintenance therapy.

Although this series is small, it is felt that methylthiouracil is a potent antithyroid agent, even in the small dosages employed. It apparently is of a low degree of toxicity, and may well represent an antithyroid drug equal to and perhaps better than propylthiouracil in clinical effectiveness.

64. Modification of the Estrual Cycle of the Ewe by the Use of Progesterone the Effect upon Subsequent Ovulation Rate and Fertility of Ova.

R. H. Dutt and L. E. Casida. Department of Genetics, University of Wisconsin, Madison.

Two groups of sheep were studied, each receiving a different dosage of progesterone. One group of 18 ewes was given 14 daily subcutaneous injections of 5 mg. of crystalline progesterone (Glidden) dissolved in corn oil; six of the animals were started on the fourth day after the beginning of natural estrus; six on the eighth day and six on the 12th day. Treatment of these different animals ended on the 18th, 22nd and 26th days, respectively after the first day of estrus. A second group of 12 ewes with four animals in each stage of the estrual cycle was treated in the same manner, except that the daily dose was 10 mg. of progesterone. The ewes were checked twice daily with an "aproned" ram for the occurrence of estrus and on the day following the last injection an observational laparotomy was performed on each ewe to study the condition of the ovaries. The ewes showing estrus after laparotomy were artificially inseminated and all ewes were slaughtered within seven days after the end of treatment.

Both dosages suppressed the appearance of estrus during treatment. Laparotomies showed that ovulation was inhibited in 15 of the 18 ewes on the 5 mg. dose, the other three having ovulated "quietly." At autopsy, two of the 15 ewes showed a cystic condition of the ovaries which had developed during the interval between laparotomy and autopsy and another had a quiet ovulation during the same period. The remaining 12 ewes in this group all showed a post-treatment estrus and ovulation within two to four days after the last injection. The ovulation rate of these ewes was significantly depressed (1.4 as compared with 1.9 for the control ovulations) and only 44 per cent of the recovered ova were fertilized.

Ovulation was inhibited during treatment in all ewes on the 10 mg. dosage and estrus of normal duration occurred in every ewe on this dosage beginning from 3 to 3.5 days after the last injection. Each of these ewes had ovulated and showed normal-appearing corpora lutea at the time of autopsy. The average ovulation rate, 1.7 per ewe, was not significantly different from that of the immediately previous control estrus, 1.8, and 67 per cent of the recovered ova were fertilized.

Thus the various estrual rhythms of different sheep were modified so that all ewes were in a potentially fertile estrus at the same time.

65. Effects of Various Estrogenic Preparations on the Vaginal Mucosa.

Mildred Vogel, Thomas H. McGavack and Joseph Mellow. From the New York Medical College Metropolitan Hospital Research Unit, Welfare Island, New York.

One hundred and fifty-one studies have been made on 78 women who were castrated or past the menopause to determine the effects of 9 different estrogenic preparations. These included estradiol in sesame oil; propylene glycol in aqueous suspension; estradiol dipropionate in sesame oil; estradiol benzoate in peanut oil, and in propylene glycol; combinations of estradiol, and estradiol benzoate in sesame oil, and in propylene glycol; and estrone, in aqueous suspension. The dosage ranged from 0.5 mg. to 5 mg of estrogen.

Vaginal smears were obtained prior to injection and at either 12 or 24-hour intervals thereafter for periods ranging from 60 to 377 hours. Whenever a patient was tested with more than one preparation, 3 weeks or more were allowed to elapse between injections.

The range of lagtime varied from 24 to 180 hours; the duration of the estrogenic response ranged from 24 to 192 hours. In most cases an inverse relation exists between lagtime and the amount of hormone injected and there is a direct correlation of duration and degree of activity with the amount of estrogen administered.

Symptomatic relief, which was experienced by 21 climacteric women occurred in most cases prior to the objective response.

66. The Use of the Vaginal Smear in the Assay of Estrogens Given Orally or Intramuscularly.

Willis E. Brown and J. T. Bradbury. Department of Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, Iowa.

The laboratory method of estrogen assay in the castrate rat has been adapted to the woman past the menopause in an effort to obtain objective evidence of relative potencies of various estrogen in women. Groups of 10 to 20 women have been used at each dose level and the dose decreased until the minimal effective dose was found. Vaginal smears were obtained once a week. The oil solutions were given as a single intramuscular injection and the oral preparations were given daily for 10 days.

Stilbestrol given orally definitely stimulated the vaginal mucosa in doses of 2.5 and 1.0 mg. per day, whereas 0.5 mg. per day gave only a minimal response. Stilbestrol given intramuscularly gave maximal response in doses of 5.0 and 2.0 mg. One milligram gave a definite response and 0.5 mg. produced no significant change in vaginal cytology.

Estrone (Theelin in oil—Parke, Davis & Co.) given orally produced maximal cornification changes at doses of 1 and 2 mg. per day; a dose of 0.5 mg. per day produced a minimal response. The minimal effective dose of Estrone intramuscularly is being determined and will be reported.

Natural estrogens given orally (Menagen—Parke, Davis & Co.) produced a vaginal response at dose levels of 20,000 I.U. and 10,000 I.U. per day that were equivalent respectively to those induced by 1 mg. and 0.5 mg. daily doses of stilbestrol. Natural estrogen (Estrogenic Substances—Smith Dorsey) were given intramuscularly in oil solutions and in aqueous suspension. The response to 20,000 I.U. was comparable to that obtained with 1.0 mg. of stilbestrol. No difference in effect could be detected between oil solutions, aqueous suspension of large crystals, or small crystals (Mixed Estrogens—Lakeside).

Trimethyl Estradiol Acetate (Lakeside) was the most potent estrogen tested, doses as low as 0.16 mg. giving a definite change in vaginal cytology.

Estriol (Parke, Davis & Co.) given in doses of 1.0 mg. orally daily for 10 days produced no detectable effect on vaginal epithelium.

67. The Similarity of Estrogenic Effect in Premenstrual Tension, Menstrual Anomalies, Chronic Cystic Mastitis and Cancer of the Breast.

Joseph H. Morton. From the New York Medical College, Flower and Fifth Avenue Hospitals, New York.

Our previous studies have indicated that an estrogen-progesterone imbalance with a relative excess of estrogen is the major etiologic factor in premenstrual tension, certain menstrual anomalies, and chronic cystic mastitis. These clinical manifestations of increased cellular proliferation and retention of intercellular fluid have been duplicated by administering estrogen to castrates and normal women past the menopause. There has also been increasing evidence in the recent literature that continued estrogen secretion accelerates epithelial neoplasia and conversely, that diminution of high endogenous estrogenic activity by castration or administration of large doses of testosterone slows the progress of the cancerous growth.

In the current study, the effect of estrogen is being investigated in patients with premenstrual tension, menometrorrhagia, chronic cystic mastitis, and carcinoma of the breast. The stimulating effect of uninhibited estrogen on epithelial proliferation is demonstrated in the breast by the presence of intracystic papillomas and cyst formation and in the uterus by a highly proliferative or mixed endometrium which is characterized clinically by shortened menstrual cycles and increased vaginal bleeding. Evidence of cyst formation in the ovary is present in some cases. Increased interstitial fluid retention resulting from estrogenic activity is manifested, depending on the part involved, by painful distention of the breast, nausea and vomiting, dysmenorrhea, abdominal distention, headaches, syncope, irritability, and occasionally localized edema.

The clinical picture is correlated with breast and endometrial biopsies and vaginal smears. The urinary excretion of estrogen, pregnandiol, and gonadotropin is assayed; determinations of calcium, potassium, sodium and sugar tolerance are made; and daily body weights and basal temperatures are recorded. Determinations of capillary permeability during and after the state of fluid retention are done by the fluorescein method.

An evaluation of the above findings and a discussion of certain implications is presented in this preliminary report.

68. Hyperestrogenism Treated with Lactogenic Hormone (Prolactin).

Manuel Villaverde. Habana, Cuba.

In this paper the hyperestrogenic syndrome is considered an actual clinical entity. Diagnosis of the condition may be accomplished either by clinical examination or by laboratory tests (biopsy or vaginal smears).

Thirty-nine cases were studied; they strictly correspond to the picture of hyperestrogenism (checked by means of vaginal smears which were dyed simultaneously with Shorr's single differential stain, and Mack's iodine vapor stain).

These patients were treated with lactogenic hormone, the action of which is antiestrogenic, luteotropic, and regulatory of hormonal balance. The patients were injected in monthly series, for one to seven series. The total dosage ranged from 720 units given in 12 days, to 2700 units given in 15, 18, or 21 days, for each series.

Good results were obtained in 25 cases (64%); fair, in 7 (18%); and there was no improvement in 7 (18%). From these 39 cases, 69 different syndromes were studied, some patients showing more than one. The results obtained were as follows:

SYNDROME	No. of Cases	RESULTS Bad	Fair	Good
Premenstrual tension	21	2 (9.5%)	4 (19.1%)	15 (71.4%)
Mastodynia	17	5 (29.4%)	2 (11.8%)	10 (58.8%)
Nervous & mental symptoms	12	2 (16.6%)	1 (8.3%)	9 (75.1%)
Dysmenorrhea (5 membranous)	7	1 (14.3%)	1 (14.3%)	5 (71.4%)
Frigidity	5	1 (20.0%)	2 (40.0%)	2 (40.0%)
Midterm symptoms	3	1 (33.3%)		2 (66.6%)
Uterine bleeding .	2		1 (50.0%)	1 (50.0%)
Migraine	1			1 (100%)
Leucorrhea	1			1 (100%)

Nervous and mental symptoms (compulsive states, mania, depression, nervousness), and premenstrual tension reacted very well to lactogenic hormone. Migraine, and leucorrhea, particularly the former disappeared. Most patients improved.

Consequently, the lactogenic hormone of the pituitary gland is an effective means to control hyperestrogenism, whatever its clinical picture may be.

69. The Factor of Rhythm in Experimental Menstruation.

Doris H. Phelps. Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine, Nashville, Tennessee.

To obtain information concerning mechanisms involved in the production of menstrual disorders, twenty-nine ovariectomized rhesus monkeys were injected with various combinations of the ovarian hormones. Observations on uterine bleeding were correlated with observations on endometrial vascular phenomena as seen in intraocular endometrial transplants. Two hundred and twenty cycles of uterine bleeding were produced, 1 to 13 per monkey. This report is one of a series dealing with various aspects of the study.

In previous reports it was shown that the duration of experimentally induced menstruation is influenced by a number of factors, among them 1) qualitative and quantitative aspects of the hormonal stimulus that produces the bleeding, 2) qualitative and quantitative aspects of hormonal treatment administered in previous experiments and 3) qualitative and quantitative variation in the hormonal stimuli acting in successive cycles. This report deals with another factor of considerable importance; it may be designated variation, in successive cycles, in the chronological relationship of estrogen action to progesterone action. The results to be described afford evidence concerning the interaction of current and previous cycles of hormonal stimulation and the interaction of hormonal and vascular mechanisms in the control of uterine bleeding. The clinical implications are discussed.

70. Hormonal Pellets in the Management of the Menopausal Syndrome.

Robert B. Greenblatt and Roland R. Suran. From the Department of Endocrinology, University of Georgia School of Medicine, Augusta, Georgia.

Patients in the menopause, because of the severity and duration of the syndrome, or lack of adequate amelioration of symptoms by usual methods of therapy, were treated by implantation of hormonal pellets. Satisfactory alleviation of symptoms was obtained in almost every instance. The method proved most convenient. The use of the Kearns pellet injector largely eliminated the disadvantages of previous methods of pellet implantation and expulsion of the pellets was a rare occurrence. The method of implantation is described in detail.

The implantation of estradiol pellets alone or in combination with pellets of progesterone should be reserved for the patient who has previously had the uterus removed, since uterine bleeding was a common complication. This undesired reaction could be obviated in most instances, however, if a pellet of testosterone was added to the one or more pellets of estradiol implanted.

It was felt that the gradual and progressive absorption of hormonal pellets of estradiol more closely approached the endogenous mechanism of ovarian hormone secretion and its decline than did the repeated administration of estrogens parenterally at varying intervals. Pellets of estradiol 25 mg. in weight were employed. These were used alone or in combination with progesterone or testosterone. A small series of patients was treated with testosterone pellets alone for comparative studies.

71. The Effect of Hypophysectomy on the Ovulability of the Ovarian Follicle of the Domestic Hen.

Irving Rothchild and R. M. Fraps. Bureau of Animal Industry, United States Department of Agriculture, Beltsville, Maryland.

Throughout the day preceding that of its normally expected ovulation, the first follicle of a hen's clutch can be ovulated by intravenous injection of minute amounts of pituitary preparations (Fraps: *Anat. Rec.* **96:** No. 4, 77, 1946). The effect of hypophysectomy on the ovulability of this follicle was studied by making intravenous injections of male-chicken pituitary at various intervals before and after hypophysectomy, on the day before expected ovulation. The dose of pituitary used (0.05-0.075 mg./bird) induced ovulation in 80 to 100% of normal intact or sham-hypophysectomized birds.

Injection at 0 or 3.0 hours after hypophysectomy resulted in ovulation of less than 10% of treated birds (4 out of 46). Injection at 6.0 or 9.0 hours after hypophysectomy, however, resulted in 40% (12 out of 30), and 46% (6 out of 13) ovulation, respectively. When injected at 1.0, 2.0, or 3.0 hours before hypophysectomy, 0% (24 birds), 36% (5 out of 14), and 93% (14 out of 15) of treated birds, respectively, ovulated.

The apparently paradoxical greater reactivity of the follicle at 6.0 and 9.0 hours than at 3.0 and 0 hours after hypophysectomy may be related to the onset of atresia, which becomes microscopically visible in some birds at 12 hours, and is well advanced in most birds at 18 hours after hypophysectomy.

The study is being continued to determine the hormone requirements, after hypophysectomy, for the maintenance of normal reactivity of the follicle to this and other ovulation-inducing gonadotropins.

72. Prognostic Value of Pregnanediol Excretion in Threatened Abortion with Special Reference to the Effects of Diethylstilbestrol.

A. R. Abarbanel. Department of Obstetrics and Gynecology, Institute of Experimental Medicine, College of Medical Evangelists, Los Angeles, California.

Excretion of pregnanediol was measured weekly in a series of women beginning with the first week of the skipped menses.

In cases of threatened abortion, diethylstilbestrol frequently stopped the bleeding and cramps. In every case where pregnanediol disappeared from the urine, the pregnancy was *not* salvaged. Where pregnanediol continued to be excreted, the pregnancy continued, except under special circumstances.

Women, early in normal pregnancy, were given from five to one thousand milligrams of diethylstilbestrol daily to form a control group. In no instance did this estrogen cause a drop or a significant rise in pregnanediol excretion.

In short, the therapeutic efficacy of stilbestrol in salvaging a threatened pregnancy can be assessed by determination of pregnanediol in the urine.

73. Further Studies on the Endometrial Cups of the Pregnant Mare.
H. H. Cole and G. H. Hart. University of California, Davis.

In a previous paper (*in* Essays in Biology, in honor of H. M. Evans, Univ. of Calif. Press, 1943, pp. 107-119) specialized glandular structures, the endometrial cups, were described and evidence was submitted supporting the view that these cups secrete equine gonadotropin, the gonadotropin in pregnant mare serum. It is the purpose of the present paper to elaborate on the description of these cups and to give an account of the allantochorionic pouches frequently associated with them.

Data for the earlier paper were obtained from mares sacrificed at known stages of pregnancy. The stage of pregnancy of the 55 cases forming the basis for the present study, was determined solely by the crown-rump (C.R.) length of the fetus.

The endometrial cups are arranged in the form of a crescent in the area of the endometrium underlying the chorion at the point where the vessels of the umbilical cord fan out to the allantochorion. The cups are present in the endometrium at the earliest stages we have studied, i.e. 3.5 cm. C.R. length. In a total of 6 cases (including 3 of the previous study) with a fetal length of 3.5 to 10 cm., the cups have been present in every instance. In 8 of 26 cases between 12 and 25 cm. C.R. length the cups were not found. As nearly as we can determine from previous studies, 12 to 25 cm. would represent a stage of pregnancy extending approximately from 85 to 150 days. Our studies indicate that the cups begin degenerating in some instances when the fetal length is 15 cm. This is approximately the time at which the hormone concentration in the serum begins to drop. The first gross evidence of cup degeneration is the development of a straw-colored appearance due presumably to a reduced blood supply. Following this, desquamation of many, if not of all cups ensues.

Allantochorionic pedunculated pouches may form at the point of contact with the endometrial cups. These pouches extending into the allantoic cavity vary in length from 3 mm. to 10.5 cm. They contain the secretion of the endometrial cups, usually amber-colored and waxy, and what appears to be the desquamated cups. If pouches do not form, the desquamated cups lie free between the endometrium and the chorion. These pouches were found in 16 of 28 cases with a fetal C.R. length of 25 to 37.5 cm.

74. Inactivation of Posterior Pituitary Antidiuretic Hormone of the Liver.
W. J. Eversole, J. H. Birnie and Robert Gaunt. From the Department of Zoology, Syracuse University, Syracuse, New York.

Numerous reports indicate that a retention of water may result from liver damage both in experimental animals and man. This has been associated with the presence of antidiuretic substances in urine and ascitic fluid (Labby and Hoagland; Leslie and Ralli).

Following Heller and Urban's demonstration that liver slices inactivate pitressin, the suggestion arose that damaged livers failed to inactivate posterior pituitary antidiuretic substances, thus causing water retention. The experiments reported here, in which the action of the liver on pitressin *in vivo* was studied, are consistent with this hypothesis.

Forty milliunits of pitressin administered subcutaneously caused a consistently greater reduction in the diuretic response to water in partially hepatectomized rats (13 cases) than in normal ones (17 cases.) Ninety minutes after water and pitressin administration the difference between the portion of water excreted by hepatectomized and by hepatectomized pitressin-treated animals was 63 per cent. This is in contrast to the difference between intact and intact pitressin-treated animals which was 47 per cent.

To test further the possibility that the normal liver *in situ* inactivates antidiuretic hormone, the effect of 40 milliunits of pitressin was studied in intact animals, using different injection routes. Intrasplenic (14 cases) or intrahepatic (6 cases) injections were markedly less effective in suppressing water diuresis than subcutaneous or intramuscular injections (20 cases). Sixty to 120 minutes after administration of water, from two to four times as much had been excreted by the animals given pitressin by an hepatic portal absorption route as by those which received the hormone subcutaneously.

These results are interpreted to indicate that the normal liver is one site of inactivation of pitressin, and that when the amount of functional liver tissue is reduced the effectiveness of the hormone is thereby enhanced.

75. The Estimation of Dehydroisoandrosterone and Related Compounds in Human Urine by a Modification of the Pettenkoffer Reaction.

Richard L. Landau and Kathleen Lugibihl. From the Department of Medicine, University of Chicago.

Munson, Jones, McCall and Gallagher have described a quantitative method utilizing the color developed with furfural and sulfuric acid for the determination of dehydroisoandrosterone. Dehydroisoandrosterone and its hydrolytic breakdown products, Δ3,5 androstadienone-17 and chlordehydroisoandrosterone, were the only chromogenic ketones known to be present in urine extracts. Certain nonketonic alcohols developed a less intense color. We have applied this technique to neutral extracts of human urine.

Normal young men excreted 4.0 to 12.0 mg. daily of chromogenic material determined as dehydroisoandrosterone (17-ketosteroids 16 to 25 mg./day). Normal young women excreted 2.5 to 5.5 mg. daily (17-ketosteroids 10 to 22 mg./day). A rough proportionality existed between the amounts of "dehydroisoandrosterone" and 17-ketosteroids in these subjects and in patients with various endocrinological disorders. Serial observations on an individual varied no more than 10 to 15% for the mean.

In a man whose 17-ketosteroid excretion rose 7 mg./day for ten days due to chorionic gonadotropin administration, the amount of material determined in crude neutral extracts, ketonic and nonketonic fractions did not vary significantly. This is in accord with the idea that dehydroisoandrosterone is not a derivative of the testis hormone.

Such estimations may be of value in the diagnosis of adrenal tumors.

76. Clinical Evaluation for 17-Ketosteroids by the Rapid Method.

T. H. McGavack, S. Kenigsberg, A. M. Shearman, and I. J. Drekter. From the New York Medical College, Metropolitan Hospital Reaserch Unit, Welfare Island, New York.

Values for 17-ketosteroids in normal people obtained by the rapid method (*Jour. Clin. Endocrinol.* **7**: 795, 1947) are in close accord with results reported by older methods. A curve of 17-ketosteroid excretion from 17 years to old age has been established for normal males in more than 300 determinations on 72 subjects. The average excretion of 18 mg. during the active period of sexual life decreases to an average of approximately 9 mg. for people in the sixth, seventh and eighth decades. Data on approximately 50 patients will be used to illustrate behavior of 17-ketosteroids in various types of endocrine diseases. In general, highest values were obtained in hyperactivity of the adrenal cortex; lowest figures in panhypopituitarism. Decrease was seen in hyperthyroidism, hypogonadism, the climacteric and myxedema. Values were slightly if at all affected in hypothyroidism with myxedema, hypertension, diabetes mellitus and diabetes insipidus. Moderate increases were observed in acromegaly and hypothalamic obesity.

77. The Application of Paper Partition Chromatography to Ketosteroids.

Robert B. Burton, Alejandro Zaffaroni, and E. Henry Keutmann. From the De-Departments of Medicine and Biochemistry of the University of Rochester School of Medicine and Dentistry.

The method of paper partition chromatography of Consden, Gordon, and Martin has been modified and applied to ketosteroids. The technique is based on the different relative solubilities of compounds in a stationary aqueous phase on paper and the moving phase of a solvent immiscible with water.

It was possible to separate mixtures of ketosteroids by first forming the Girard "T" derivatives and chromatographing the latter with water-saturated 1-butanol. The positions of the compounds were determined by developing a color with Kraut's reagent or a solution of platinum iodide. The monoketones as a group were widely separated from the diketones. The number of other polar groups also influenced the positions of the spots but to a lesser extent.

The mean Rf values $\frac{\text{(distance spot has moved)}}{\text{(distance solvent has moved)}}$ of the ketosteroids available to us were found to be as follows:

Testosterone Androsterone Dehydroisandrosterone	Rf = 0.57
Estrone	Rf = 0.53
Δ4 pregnenetriol-17,20,21 one-3	Rf = 0.40
Progesterone	Rf = 0.16
Δ4 Androstenedione-3,17	Rf = 0.12
17-hydroxyprogesterone	Rf = 0.10

Desoxycorticosterone and 11-dehydro-17-hydroxycorticosterone in their free forms were chromatographed with reproducible results using water-saturated 2-ethylhexanol. The positions of the compounds were determined with alkaline silver reagent or Jaffe's reaction. The same procedure was applied to chloroform concentrates of commercial aqueous adrenal cortex extracts, to neutral ethyl-acetate solids of pork adrenal cortex extract, and to neutral extracts of pooled urine from patients in alarm reaction:

Desoxycorticosterone	Rf = 0.92
11-dehydro-17-hydroxycorticosterone	Rf = 0.87
Aqueous A.C.E.	Rf = 0.00; 0.33; 0.47; 0.60; 0.82
Pork adrenal cortex solids	Rf = 0.00; 0.34; 0.63; 0.79
Neutral urine extract	Rf = 0.00; 0.76

Pooled extracts of the migrating spots obtained by chromatographing aqueous adrenal cortex extract, when injected into mice, exhibited glycogenic activity approximately equal to the original sample. The activity of individual spots is not being determined

78. A Rapid Modification of the Zimmermann Test for Ketosteroids.

Sidney Pearson and Sylvester Giaccone. From the New York Medical College Metropolitan Hospital Research Unit, Welfare Island, New York.

A modification of the Zimmermann test for ketosteroids is described. Solutions are kept at 25 degrees C. for 20 minutes instead of 60 to 90 minutes as in other methods. A

thermostatically controlled water bath and exclusion of light are unnecessary, and the final color is stable from 40 to 60 minutes. As many as 100 determinations can be completed in one hour. The procedure is not as subject to error as the previous method (*Jour. Clin. Endocrinol.* **7**: 795, 1947), because it is not as greatly influenced by impurities in the reagent or the extract. Moreover, more color is developed per unit weight of ketosteroids.

In this procedure the dry ketosteroid is taken up in 0.4 ml. of a 1.0% absolute alcohol solution of metadinitrobenzene and 0.3 ml. of 8N aqueous KOH are added. The solution is placed in a water bath previously adjusted to 25 degrees C. After 20 minutes, one ml. of an alcohol-water (3:1) solution is added and the solution read in the colorimeter.

One hundred and fifty-eight determinations on normal and pathological urines were made using the procedure described and the procedure which requires 90 minutes of heating at 30 degrees C. with 5N aqueous KOH. The average deviation between the two procedures in the 24-hour excretion was 1.5 mg. equivalents dehydroisandrosterone.

79. A Fluorometric Method for the Determination of Estrone and Estradiol in Human Urines.

Joseph W. Jailer. Department of Medicine, Obstetrics and Gynecology and Anatomy, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York 32, N. Y.

In a preliminary paper (Jailer: *Endocrinology* **41**: 198, 1947) data were presented to show that when sulfuric acid is added to an alcoholic solution containing estrone or estradiol, a yellowish-green fluorescent substance results. Use was made of this phenomenon to develop a quantitative fluorometric method to determine minute (0.05–0.3 micrograms) amounts of estrone and estradiol in human urine. The extraction procedure of Bachman and Petit has been followed for the preparation of the urinary extract. Data are presented to show the reliability of this method for the clinical determination of urinary estrogens. This evidence comes in the following categories: 1) Recovery of known amounts of estrogen added to urine, consistently averaging from 50–60%. 2) Urinary excretion of estrogens has been shown to be high in certain clinical conditions and low in others. Results obtained with this method compare with those reported in the literature as performed with the bioassay. 3) Parenteral administration of estrogens resulted in an increased excretion of this fluorophore in the urine. 4) Values obtained with biological assay and with the chemical method have been compared in urine from both humans and stallions, and have been shown to be of the same order of magnitude.

80. A Clinical Bio-Assay for Chorionic Gonadotropin.

A. Albert. Mayo Clinic, Rochester, Minnesota.

None of the numerous methods for bio-assay of chorionic gonadotropin in clinical use possesses all of the following attributes: speed, convenience, a reasonable but known error, and a universally understood unit of measurement. The following method was designed to approximate such attributes.

Immature rats of uniform age, sex, weight and strain are used. The end point is ovarian hyperemia. The kinetics of response to single subcutaneous doses (0.25–100 I.U.) of the International Standard indicate a fairly steady state within four hours. At this fixed interval, a regression line was fitted for dosages between 0.5 and 5.I.U. The parameters of this line have remained constant. Analysis of the dose-effect curves and tests of the fiducial limits indicate an over-all error within ± 30 per cent.

Urinary constituents have no effect on the assay but there is a marked "protein effect" in serum assays. Consequently, urine is injected undiluted and at various log dose intervals =0.4771. Serum is diluted 1:10 to avoid the "protein effect," and then at similar log dose intervals. No chemical or physical concentration is necessary.

Representative values of chorionic gonadotropin concentration in various clinical states and conversion factors for older methods are presented.

81. Specificity of a Colorimetric Method for Dehydroisoandrosterone in Urine Extracts.

Paul L. Munson, Mary Ellen Jones (by invitation), Philip J. McCall (by invitation), and T. F. Gallagher (by invitation). From the Department of Biochemistry, University of Chicago, and the Pituitary Research Section, Biochemical Research Department, Armour and Company.

Dehydroisoandrosterone (DHA), both in pure solution and urine extracts, may be estimated by a simple method based on the color produced when acetic acid solutions containing 10 to 50 gammas of the steroid are treated at 67° C. with furfural and sulfuric acid.

Fifty-six different crystalline neutral steroid compounds have been tested. Forty compounds gave no significant color, and with the exception of DHA and its acetate and sulfate, only 4 neutral ketones gave positive tests. Two of these, $\Delta^5$3(β)-chloroandrostenone-17 and $\Delta^{3,5}$-anrostadienone-17, are generally considered to be transformation products derived from DHA during the customary acid hydrolysis of urine. So far as is known, the remaining two positive ketones, pregnanolone and 21-acetoxy-pregnenolone, do not occur in urine. Nine neutral nonketonic unsaturated alcoholic steroids reacted to give varying color intensities. In general, the test indicates the presence of unsaturation in Ring B and a hydroxyl group or double bond in Ring A, but is not completely specific for these functional groups.

It is concluded that the methods will provide a serviceable estimate of the amount of DHA (and its transformation products) in ketonic urine extract fractions, and may be of assistance in investigations of the relatively unexplored alcoholic nonketonic fraction.

82. The Colorimetric Determination of Sodium and Its Application in the Study of Sodium and Chloride Balance.

Joseph W. Goldzieher and Gilbert Stone. From the Endocrine Division, St. Clare's Hospital, New York and the Department of Biochemistry, the City College of New York.

A new colorimetric method for the determination of sodium is presented. Sodium is precipitated and purified as sodium uranyl zinc acetate. Reliable color intensification is achieved by conversion to a deeply colored ion, presumably hydrated uranium tetroxide. The method has a sensitivity of better than 4 per cent.

Balance studies were carried out on a series of patients given 10 Gm. of sodium chloride after a control observation period. Only a small percentage of the cases show a parallelism between the excretion of sodium and chloride, whereas marked sodium retention without chloride retention may occur. These findings suggest that chloride studies cannot be used to investigate sodium metabolism, and that earlier work based on such methods may need re-evaluation.

83. Depression of Lymphocyte Count After Orally Administered Glucose.

Paul A. Marks, Dorothy T. Marks and Joseph W. Jailer. Departments of Medicine, Obstetrics and Gynecology and Anatomy, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York, N. Y.

It has been established that the injection of A.C.T.H. and/or adrenal cortical hormone results in a decrease in circulating lymphocytes and a dissolution of lymphoid tissue. Pincus and his collaborators have shown that exposure to stress will cause a diminution in circulating lymphocytes and an increase in urinary 17-ketosteroids. One of the types of stress used by these investigators was the glucose tolerance test. It seemed to be of interest to ascertain whether there was any difference between diabetic and nondiabetic patients in the response of circulating lymphocytes to the routine oral glucose tolerance tests. In normal individuals it was found that after the oral administration of 200 Gm. of glucose, the average maximum decrease was 20% of the control fasting value. In diabetics (not receiving insulin) the average maximum fall was 42%. In one patient with Cushing's syndrome, there was a maximum fall of 45% from the control value although the blood glucose response was but mildly diabetic. In another with Simmonds' disease, intravenous glucose caused but a minimal fall in the circulating lymphocyte count. The mechanism involved is discussed.

84. Pitfalls in the Diagnosis of Diabetes.

Bernard A. Watson. Clifton Springs Sanitarium and Clinic, Clifton Springs, N. Y.

1) The incidence of glycosuria found during routine physical examinations of 51,096 college students was 1.14% (601 subjects). Standard dextrose tolerance tests on these 601 subjects revealed approximately 15% or 93 gave values which were significantly above normal as judged by figures obtained in tolerance tests on 83 healthy young students (Fig. 1).

	Fasting	½ hr. after 100 Gm. glucose	1 hr.	2 hrs.	2½ hrs.
Mean	92	135	115	91	83
Upper range 90% cases	117	184	174	120	119

Fig. 1

2) Seventeen years' experience has shown the inaccuracy of diagnosing diabetes on the basis of a single elevated dextrose tolerance test in the absence of clinical signs or symptoms. Subjects having mildly disturbed tests should not be put on a diet, but rechecked by periodic tolerance tests at intervals of 3 to 6 months to determine whether only temporary hyperglycemia existed or whether there was a trend toward clinical diabetes. A thorough history and physical examination to determine whether or not other causes of hyperglycemia are present must be done in all cases.

3) The error of accepting recently published standards for diagnosis of diabetes (Diabetes in a New England Town, by Wilkerson and Krall) will be demonstrated by actual case histories. The necessity for requiring glycosuria is questioned as it was found in 12% of our normal subjects (Fig. 1).

4) A simple mathematical formula is proposed which, applied to a single tolerance test, will aid in predicting whether in the presence of a mild disturbance the patient should have further observation and/or dietary restriction.

85. Electrolyte Balance Studies in the Uncontrolled and Controlled Diabetic State.

Jonas Weissberg, Thomas H. McGavack, A. M. Shearman and I. J. Drekter. From the New York Medical College Metropolitan Hospital Research Unit, Welfare Island, New York.

In order to evaluate the changes in electrolyte balance during the uncontrolled and controlled diabetic state, six patients with diabetes mellitus were allowed to develop ketosis by reducing their intake of insulin. Prior to and following such a decrease, these patients were given an analyzed liquid diet.

The total urinary output of nitrogen, sodium, potassium and chloride was measured in each subject. During control periods such determinations were made on 8-hour aliquots. During periods of severe ketosis and just prior to and following the inauguration of insulin and other therapy, these analyses were obtained for 2-hourly urinary fractions. During control periods a number of determinations of serum potassium, sodium, chloride, and phosphate were made in each instance. After severe ketosis had been established, these were performed several times daily.

In five additional patients, who entered the hospital in diabetic acidosis, the above studies were duplicated, except that no control could be established.

As compared with the control, the state of ketosis showed a) a slight increase in sodium and potassium excretion; b) no significant change in chloride excretion; c) no changes in amounts of other urinary constituents or in levels in blood serum of any of the substances previously mentioned.

There was no significant variation in nitrogen balance for any subject during any of the three periods. As compared with the control and ketosis periods, the period of therapeutic response showed a significant fall in serum potassium in 10 of 11 subjects. The movements of phosphate in the blood serum closely paralleled those of potassium. During the state of ketosis, there was always a negative balance for potassium.

86. The Role of the Endocrine Glands in Body Temperature Regulation.

H. E. Nieburgs and Robert B. Greenblatt, University of Georgia School of Medicine, Augusta, Ga.

Body temperature depends on the balance between the heat of oxidation and the loss of heat from the body. A contributing role of the hormones in this process is suggested by body temperature changes in relation to the menstrual cycle in women. The low temperature during the follicular phase, with or without a further drop at ovulation, followed by a higher temperature level during the luteal phase, appears to be due to the action of estrogen and progesterone respectively. The administration of these hormones in high enough doses in humans produces similar effects.

The effect of hormones on body temperature changes was investigated in five experimental series on a total of 108 rats. In intact female rats body temperature shows a characteristic pattern in relation to the five-day cycle, with a drop at or following estrus. Endogenous progesterone activity in the rat with pseudopregnancy maintains a high temperature level.

In castrated female rats the administration of progesterone increases the temperature above that of castrated controls and estrogen produces a decrease of temperature below that of the control animals. When testosterone propionate was injected in addition to estrogen a further decrease of temperature was obtained. Contrary results, however, may be obtained under different experimental conditions.

Extract of the adrenal cortex has the tendency to keep the temperature on a low level, slightly above that of castrated control animals. Thymus extract increases body temperature above that of castrated controls but maintains the temperature below that of animals treated with progesterone.

The same results, though less definite, were obtained in thyroidectomized animals. In adrenalectomized, ovariectomized rats, however, estrogen increases the temperature above that of the controls and progesterone causes a further increase.

Adrenalectomy in addition to ovariectomy produces a lower temperature than ovariectomy alone, whereas thyroidectomized-ovariectomized animals show the lowest temperature. In cases of both estrogen and progesterone administration this relationship changes to the effect that the adrenalectomized-ovariectomized animals show the highest temperature level, whereas a lower level is shown by the castrated controls; and the lowest level is seen in thyroidectomized-ovariectomized animals.

It thus appears that hormones influence body temperature and that the adrenal and thyroid gland change the effect of estrogen and progesterone on body temperature to a certain degree. The possible mechanism of action is discussed.

87. The Lipogenic Activity of Propylthiouracil, Triphenylchloroethylene and Hexestrol in Chickens.

R. George Jaap. Ohio State University.

Previous research has demonstrated that 0.1 to 0.2 per cent thiouracil in the diet of growing chickens produces the optimum lipogenic effect with a minimum retardation of growth rate. Propylthiouracil was fed at 0.02, 0.066 and 0.20 per cent of the ration for a four-week period to thirteen-week-old crossbred (White Leghorn × Rhode Island Red) cockerels. At the lowest dosage, gain in weight was reduced from 1.30 to 0.89 pounds and abdominal fat increased from 15.5 to 36.3 grams. On the higher doses growth rate was further reduced with no appreciable increase in fatness. It would appear that propylthiouracil is approximately ten times as potent as thiouracil in promoting lipogenesis in growing chickens.

Previous tests have shown that triphenylchloroethylene, on oral administration, has slight estrogenic properties on both chickens and turkeys; and, in the latter, has a lipogenic effect without the excessive lipemia characteristic of the more active estrogen, dianisylhexene. Tests with a similar group of cockerels as described above have demonstrated that the triphenylchloroethylene at oral dosages at 0.01 and 0.02 per cent of the diet depress testis size but promote only slight increases in fat deposition.

The subcutaneously-implanted fused pellet of diethylstilbestrol frequently fails to suppress maleness in mature cocks and, in these instances, is relatively ineffective in promoting fat deposition. Tests with hexestrol in a fused pellet of similar size (15 to 20 mg.) indicate that it is much more effective in suppressing testis activity and produces greater fat deposition in mature cocks. In the more comprehensive of these tests, hexestrol-treated cocks had an average of approximately twice as much abdominal fat and one-half the testis weight of those receiving diethylstilbestrol.

88. The Influence of the Pituitary Gland on Blood Vessel Development. The Effect of Concentrated Pituitary Extract on the Isolated Kidney.

Robert C. Moehlig and Louis Jaffe. From the Department of Internal Medicine and Clinical Investigation, Harper Hospital, Detroit, Michigan.

The purpose of the investigation was to show that the pituitary influences the development of the blood vessels. Other work has shown that this gland influences hemopoiesis.

Since an arteriovenous fistula of the hand produces an abnormal growth by lengthening the bones, and since there is no abnormal amount of growth hormone present in the hand it was felt that growth was produced by the augmented blood vessel development and increased oxygenation in the affected hand. What further strengthened our belief that the pituitary influenced blood vessel development was the vasoconstrictor effect of the posterior lobe secretion. This led to the hypothesis advanced by Krogh that the posterior lobe maintains capillary tone, thus giving the whole pituitary gland a major role in vascular system development and control.

In order to test the validity of this assumption we used a physiologically potent whole pituitary from a sheep. This product was supplied in a relatively high concentration to one of the two kidneys of a dog, thereby permitting the other kidney to act as a control. The results of these studies are reported.

89. Results of Prolonged Medical Treatment of Obesity with Diet Alone, Diet and Thyroid Preparations, and Diet and Amphetamine.

David Adlersberg and Martin E. Mayer. The Mount Sinai Hospital, New York, N. Y.

The study extends over a period of 5½ years and presents the results of treatment of obesity under controlled conditions in the Nutrition Clinic of a large general hospital. It includes 289 patients, 27 men and 262 women. Moderate uncomplicated hypertension without any evidence of heart failure was present in about 25% of the individuals. Other forms of cardiovascular disease, as well as diabetes, were excluded. Each patient remained under regular observation by the same physician at intervals of 1 to 4 weeks throughout the entire period of therapy.

The patients were classified according to age, sex and degree of overweight (before treatment was initiated). Class I represented those with an overweight of 1–20%; class II, 21–40%; class III, 41–60%; class IV, 61–80%; and class V, over 80%. The majority of the patients were in classes II and III.

Treatment consisted in 132 patients of a low calorie diet (approximately 1200 cal.), without any additional medication over a period of 5.5 to 11.7 months (group A). In 61 patients, a preliminary period of diet alone was given for 2.2 to 4.6 months; this was followed by a second period of 5 to 6.7 months during which time the same diet was combined with oral thyroid medication, usually 1 to 4 grains of desiccated thyroid daily (group B). In 80 patients, a preliminary period of diet alone was given for 1.2 to 5.2 months; this was followed by a second period of 2.7 to 11.8 months in which the same diet was combined with oral administration of 5 to 10 mg. amphetamine sulphate 1 to 2 hours before lunch and dinner (group C). A final group of 16 patients went successively through diet treatment alone, diet and thyroid medication, diet and amphetamine medication, in periods of 0.75 to 27 months (group D).

The average monthly loss of weight was calculated for each group and class of patients. The results of Classes I–III are presented in Table 1.

TABLE 1

Average monthly weight loss in lbs.

Weight Class	Group A	Group B		Group C	
		Diet Alone	Diet & Thyroid	Diet Alone	Diet & Amphet.
I	0.5	1.3	0.4	0.3	1.1
II	1.0	1.2	0.9	1.7	1.8
III	1.2	1.3	0.6	1.5	1.7

It is evident that wide fluctuations were encountered in the various groups. In group A loss of weight increased with the degree of obesity; this however was not apparent in the "Diet alone"-periods of group B and C. Diet alone resulted in better weight reduction than diet plus thyroid, in group B; on the other hand, diet plus amphetamine showed for the entire group C somewhat better results than diet alone. In the latter group there were striking appetite-reducing effects of amphetamine with considerable loss of weight. The initial effect often faded under continued administration of the drug so that later increased food intake and gain in weight resulted. It is imperative in studies of this type to separate strictly the short-term effects from the long-range results.

The over-all picture of these controlled long-term observations casts considerable doubt on the therapeutic value of the drugs employed and emphasizes dietary control as the most important factor in the treatment of obesity.

90. THE URETHRAL SMEAR IN THE NORMAL HUMAN MALE.

Mildred T. Vogel, Thomas H. McGavack and Henry Kammandel. From the New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York, N. Y.

Daily urethral smears were taken by 12 men, aged 18 to 32 years, for periods of time ranging from 7 to 122 days; by 6 men, aged 44 to 58 years, for periods of time ranging from 3 to 50 days; and on 5 boys, 9 to 11 years old, every other day for one week.

The constituents of the 1056 urethral smears studied included cornified, superficial and intermediate cells derived from squamous epithelium and structures believed to be basal cells of the lower epithelial layers, which we termed round cells. The superficial, intermediate and round cells exhibit both acidophilic and basophilic staining properties. Leucocytes, bacteria, occasionally spermatozoa and certain refractile bodies of unknown nature and origin were also present. A moderate amount of debris, believed to be of cellular origin, was observed in all smears.

There was great variation present in the types of cells and in their staining properties, from person to person and in the same individual from time to time.

The urethral smears of the subjects ranging in age from 18 to 32 showed a somewhat higher degree of cornification and acidophilism than did those of the younger and older males. Moreover, the percentage of round cells and leucocytes was somewhat higher in this group.

No regularly cyclic variations in the urethral smear have been observed in any individual or in any age group.

The histology of the urethral smear and the factors which influence it are discussed.

Tables of results and photomicrographs are included.

91. THE SPECIFIC OVARIAN HYPEREMIA-INDUCING EFFECT OF LUTEINIZING AND LUTEOTROPIC HORMONES.

Herbert S. Kupperman, W. H. McShan and Roland K. Meyer. University of Georgia School of Medicine, Augusta, Ga.; Department of Therapeutics, New York University College of Medicine, New York, N. Y.; and Zoological Laboratory University of Wisconsin, Madison, Wisconsin.

Ovarian hyperemia has been employed as a definitive endpoint in the rapid (2–6 hours) pregnancy tests utilizing the rat as the test animal. Studies were undertaken to ascertain the gonadotropic factor responsible for evoking ovarian hyperemia. The following preparations from pituitary glands, serum and urine were tested: Unfractionated pituitary extracts, fractionated pituitary preparations—namely follicle-stimulating hormone, luteinizing hormone, lactogenic and thyrotropic extracts—pregnant mare serum, and pregnancy urine extracts. Some pituitary preparations were administered as aqueous suspensions of dried pituitary powder. Only those preparations exhibiting luteinizing or luteotropic activity were capable of inducing ovarian hyperemia when administered according to the described procedure. A comparison between the hyperemic action and the gonadotropic potency of the various extracts was made. In view of the specificity of the luteinizing and the luteotropic hormones in evoking ovarian hypermia, this endpoint has been employed as a rapid procedure in screening gonadotropic compounds for their luteinizing and luteotropic effects. An ovarian hyperemia unit is described and evaluation of various gonadotropins according to this unit is discussed.

PROGRAM OF THE 1948 LAURENTIAN HORMONE CONFERENCE

Forest Hills Hotel, Franconia, New Hampshire
September 13-18

Because of limited accommodations, attendance is by invitation. Correspondence should be addressed to: Dr. Gregory Pincus, Chairman, Committee on Arrangements, 222 Maple Avenue, Shrewsbury, Massachusetts.

I. STEROID HORMONE METABOLISM "IN VIVO" AND "IN VITRO"

Some aspects of Progesterone Metabolism
DR. G. F. MARRIAN, *University of Edinburgh*
Monday Evening, September 13

Recent Developments in Our Knowledge of Estrogen Metabolism
DR. R. D. H. HEARD and MRS. J. C. SAFFRAN, *McGill University*
Tuesday Morning, September 14

The Metabolism of Androgens by Tissues
DR. LEO T. SAMUELS, *University of Utah*
Tuesday Morning, September 14

The Metabolism of Estrogens with Particular Emphasis on Clinical Aspects of Physiology and Function of Ovarian Hormones
DR. ALBERT SEGALOFF, *Alton Ochsner Medical Foundation*
Tuesday Evening, September 14

II. THE ROLE OF HORMONES IN TISSUE AND BODY METABOLISM

The Antihormone Problem in Endocrine Therapy
DR. JAMES H. LEATHEM, *Rutgers University*
Wednesday Morning, September 15

Integration of the Metabolic Effects of Adrenal Cortical, Thyroid and Growth Hormones
DR. ABRAHAM WHITE, *University of California at Los Angeles*
Wednesday Morning, September 15

The Alterations in Metabolism Incident to Administration of Insulin, Adrenalin and Thyroid Substances, Studied with the Aid of Isotopes

Dr. DeWitt Stetten, Jr., *Harvard University Medical School*

Wednesday Evening, September 15

The Pancreas as the Guardian of the Liver

Dr. Charles H. Best, *University of Toronto*

Thursday Morning September 16

Metabolic Changes in Man Following Adrenal and Pituitary Hormone Therapy

Dr. George W. Thorn, *Harvard University Medical School*

Thursday Morning, September 16

III. NEUROHUMORAL-HYPOTHALAMIC RELATIONSHIPS

Adrenal Function in Mental Disease

Dr. Gregory Pincus, Dr. Hudson Hoagland, Dr. Harry Freeman and Mr. Fred Elmadjian, *Worcester Foundation for Experimental Biology*

Thursday Evening, September 16

Manifestations of Altered Autonomic and Humoral Functions in Psychoneuroses

Dr. Robert A. Cleghorn, and Dr. B. F. Graham, *McGill University*

Friday Morning, September 17

Effects of Hypothalamic Lesions on Water and Energy Metabolism in the Rat

Dr. James A. F. Stevenson, *Yale University*

Friday Morning, September 17

IV. THYROID PHYSIOLOGY AND FUNCTION

Physiologic Reactions of the Thyroid Stimulating Hormone

Dr. Rulon W. Rawson and Dr. William L. Money, *Massachusetts General Hospital*

Friday Evening, September 17

The Metabolism of Iodine as Disclosed by the Use of Radioiodine (I131)

Dr. F. R. Keating, Jr., and Dr. Alexander Albert, *The Mayo Clinic*

Saturday Morning, September 18

Radioiodine as a Diagnostic and Therapeutic Tool in Clinical Medicine

Dr. S. M. Seidlin, *The Montefiore Hospital*

Saturday Morning, September 18

Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor: ROY HERTZ. *Collaborators:* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

GONADS

WATSON, C. J.; SCHULTZ, A. L., and WIKOFF, H. M.: Purpura following estrogen therapy, with particular reference to hypersensitiviity to (diethyl) stilbestrol and with a note on the possible relationship of purpura to endogenous estrogens, *J. Lab. & Clin. Med.* **32**: 606–617, 1947.

Five cases are reported in which purpura followed the administration of estrogens. One patient was a man undergoing treatment for malignancy of the prostate, with 3 mg. of stilbestrol daily. Of the 4 women, 3 were postmenopausal, and the fourth was a 23-year-old individual with menstrual irregularities. The last mentioned had taken considerable quantities of acetophenetidine and aspirin for the relief of headaches, and had had a relatively small amount of estrogen parenterally over a period of two months prior to the appearance of purpura. In the other patients, periods of treatment, chiefly with stilbestrol, varied from 2.5 to 7 years. In 3 patients, tests for skin sensitivity to stilbestrol and estrone were completed. Twenty-five normal subjects or patients without blood dyscrasia were similarly tested as controls. In each instance, 0.1 cc. of a 0.2 per cent suspension of the estrogen in physiological saline was intradermally injected; a similar amount of vehicle was used on the opposite arm as a control. One patient reacted positively to both estrone and stilbestrol. Each of the remaining patients responded to one or the other of the estrogens. The reaction consisted entirely of an erythematous response; wheals were not observed. None of the control subjects showed any reaction. Passive transfer of the sensitivity by the method of Prausnitz-Kustner was unsuccessful in all instances, leading the authors to question the role of circulating antibody in the production of the dermic reaction. While recognizing the inconclusiveness of the data, they believe the probability exists that endogeneous estrogen is frequently responsible for certain types of purpuric phenomena. Some of the literature relating estrogens to such an hypothesis is critically reviewed.—*T. H. McG.*

PANCREAS

BLOTNER, H.: Sugar tolerance tests—methods and evaluation, *Am. J. Digest. Dis.* **14**: 245–258, 1947.

The author studied the glucose tolerance tests of 479 Army selectees who exhibited reducing substances in the urine by Benedict's method. One hundred grams of glucose

were given orally after an overnight fast and blood samples were taken one-half, one, two, and three hours later for blood sugar measurements (Folin-Wu method). Those subjects were considered normal whose fasting blood sugar was less than 130 mg. per cent and whose peak blood sugar one-half hour after the oral glucose was less than 165 mg. per cent. Potential diabetics were those whose blood sugar was more than 165 mg. per cent one-half hour or one hour after the glucose administration and who demonstrated some glycosuria during the test. A blood sugar greater than 170 mg. per cent one-half hour or one hour following the glucose indicated diabetes. Of the 479 men studied, 251 were considered diabetic, 38 were considered potential diabetics, and 190 were judged normal by the criteria mentioned. Forty-five of 114 normals whose peak blood sugar was less than 140 to 150 mg. per cent demonstrated glycosuria. Two hundred and twelve of the 251 subjects with diabetic curves had normal fasting blood sugars. Thirty-three of 367 selectees with mellituria were considered to have renal glycosuria. Subjects who had low caloric intake, low carbohydrate or high fat diet prior to the test, exhibited diabetic tolerance curves. When they were given a high carbohydrate diet, the curves returned to normal. Normal subjects given insulin had higher blood sugars in the one-half hour or one hour samples after the administration of glucose. Physical inactivity in adults led to a decreased glucose tolerance with no glycosuria at high blood sugar levels. In children, the decrease in glucose tolerance was not so severe and some glycosuria was evident.—*H.S.G.*

CONN, J. W.; HINERMAN, D. L., and BUXTON, R. W.: Effects of alloxan upon the human pancreas, *J. Lab. & Clin. Med.* **32**: 347–348, 1947.

For nine consecutive days a patient with a secreting pancreatic insulinoma was given alloxan intravenously in doses of 50 mg. per Kg. of body weight for each of the first 2 days, and 100 mg. per Kg. for each of the remaining seven days. Fifteen days after the last injection, the adenoma was removed along with a section of pancreas outside the tumor area. Microscopic examination of these tissues failed to show any recognizable effect of the alloxan upon the islet cells of the neoplasm, but did reveal marked destruction of the islets of the pancreas proper, similar in every respect to changes which have been repeatedly demonstrated in alloxan-treated laboratory animals. Severe diabetes followed the removal of the adenoma, which, while still present, had markedly decreased in severity by the sixty-fifth postoperative day. It was not believed that the histologic changes observed could have been due to the pretreatment of the patient with excessive carbohydrate ingestion, as glucose infusions had not been used, and every effort had been made to control the diet.—*T. H. McG.*

DUNSKY, J., and LAWRENCE, G.: Renal glycosuria associated with pentosuria, *J. Ped.* **30**: 416, 1947.

The authors point out that instances of renal glycosuria associated with pentosuria are infrequently recorded. They suggest that this is probably due to the fact that the possibility that a combination of different sugars is being excreted is not frequently considered. They report a case in which renal glycosuria and intermittent pentosuria occurred together in the same patient, who presented symptoms of diabetes mellitus. Laboratory findings are described.—*E.C.R., Jr.*

JONES, G. M.: Posthypoglycemic encephalopathy: case reports, *Am. J. M. Sc.* **213**: 206, 1947.

The author reports the case histories of three diabetic patients who developed per-

sonality, mental, and neurologic changes following hypoglycemia. These changes persisted after the blood sugar levels had become normal. The first patient showed mental confusion and personality changes for 48 hours after cessation of hypoglycemia; the second exhibited marked mental impairment and bilaterally positive Babinsky sign which persisted for 18 days until his death; and the third had coma and convulsions for 24 hours after the hypoglycemia was satisfactorily controlled and then died. Complete postmortem examinations of the last two patients are reported; the brain tissue showed hyperemia, edema, and diffuse necrosis. The author reviews similar cases in the literature, and discusses the physiological and pathological changes responsible for the clinical behavior. It is recommended that the term posthypoglycemic encephalopathy be used for this definite clinical entity. The author urges physicians to be cognizant of this very serious complication of hypoglycemia and to combat any prolonged low blood sugar level vigorously.—*E.C.R., Jr.*

LEWIS, L. A.; MOSES, J., and SCHNEIDER, R. W.: Plasma proteins. II. Alteration in alloxan diabetic rabbits especially in relation to ocular damage, *Am. J. M. Sc.* **213**: 214, 1947.

Plasma protein fractionations by the Tiselius technique were made on 24 rabbits before and after the development of alloxan diabetes. The purpose of this study was to determine what modification, if any, occurs in the plasma protein fractions, and to note the progress of these changes with different combinations of insulin, diet and vitamins. During these studies the eyes were examined for changes in the lens and retina. Protocols of three of the rabbits are presented in tabular form. In severe diabetes with acidosis there was a marked increase in the β-globulin and a decrease in the albumin level. With disappearance of acidosis, either following insulin treatment or spontaneous adjustment, the β-globulin level became normal. In some cases with insulin therapy, the albumin approached the normal value. In mild diabetes, without acidosis, there was frequently a decrease in the total plasma protein, but little change in the relative percentage of the various fractions. Twenty-one of the rabbits showed progressive lens changes and eight showed minute capillary bleeding in the retina. The plasma albumin was usually markedly decreased and the β-globulin increased at the time that hemorrhages were observed. Plasmapheresis accelerated the development of retinal changes. The interval before appearance of eye changes varied greatly from animal to animal and seemed to have little correlation with the severity of the diabetes, the loss of weight, or the diet. One rabbit, which failed to develop diabetes following injection of alloxan, also failed to show any plasma protein changes or eye abnormality. The authors conclude that the plasma protein changes which occur in the rabbit with alloxan diabetes are similar to those which are observed in human diabetes. The development of ocular complications in rabbits demonstrates a further similarity between diabetes mellitus in man and diabetes produced by alloxan in animals.—*E.C.R., Jr.*

LOMBARD, H. L., and JOSLIN, E. P.: Certification of death of 1000 diabetic patients, *Am. J. Digest. Dis.* **14**: 275–278, 1947.

The upward trend of deaths due to diabetes mellitus in Massachusetts was halted for two years after the introduction of insulin. Similarly the entry of protamine zinc insulin into the therapy of diabetes temporarily stopped the upward trend of diabetic deaths. The mean age of death due to diabetes in Massachusetts was 52.0 years in 1900. In 1945 it had risen to 66.8. The calculated morbidity due to diabetes in that state is 5.0 per thousand. The 1000 case records studied were taken from the files of Dr. Joslin covering

the period 1936–43. The mean age at the onset of diabetes was 55 years. The mean age at death was 66. Of the deaths in the series, those due to diabetes per se or in which diabetes was the contributing cause accounted for 64 per cent. Deaths due to intercurrent disease in which diabetes did not play a major role amounted to 11 per cent. In 24 per cent of the deaths, diabetes was not mentioned in the death certificate.—*H.S.G.*

LOWELL, F. C.: Immulogic studies in insulin resistance. III. Measurement of an insulin antagonist in the serum of an insulin-resistant patient by the blood sugar curve method in mice, *J. Clin. Investigation* **26**: 57–63, 1947.

The anti-insulin action of the serum of a previously reported case of diabetes with insulin resistance, is compared with that of the sera from a normal subject and a patient with uncomplicated diabetes, respectively. Previous methods of detecting insulin antagonists in the blood of the insulin-resistant diabetic have been mainly qualitative in character, lacking the sensitivity necessary to detect any but the largest amounts of insulin antagonist. The present method depends upon determination of the blood sugar of white mice in the fasting state and at 30 and 60 minutes after the injection of varying amounts of serum with a standard dose of insulin, after injection of a fixed amount of serum with a varied dose of insulin. For each individual determination, six mice were employed. As a rule, a rise in blood sugar occurred in those "control" animals which were injected with a mixture of normal or "uncomplicated diabetic" serum and saline. Only when an appreciable decrease in the value for glucose in the blood of the animals occurred following injection was the test considered positive. By this method, a rough quantitative estimate of the patient's actual resistance could be obtained. When the patient's serum was diluted, one part to 24 parts of saline, representing about 0.005 ml. of serum, it completely antagonized the action of 0.005 unit of insulin, an amount capable of causing profound hypoglycemia in the "unprotected" animal. On this basis, it was estimated that the patient should require from 3000 to 4000 units of insulin daily to reduce glycosuria and hyperglycemia. The author believes this type of insulin resistance is due to an "insulin-neutralizing antibody" which is sharply distinguishable from the type of antibody that gives rise to allergic cutaneous phenomena in conjunction with each injection of the hormone. The insulin antagonist found in the patient studied was most active when injected into mice simultaneously with insulin. This leads the author to conclude that it is not related to the glycotropic factor of the pituitary, which would require several hours to exert a maximal anti-insulin activity.—*T.H. McG.*

MAY, L. M., and GREGORY, R.: The effects of inflammation on the severity of diabetes, *J. Lab. & Clin. Med.* **32**: 1540, 1947.

Intense inflammatory reactions were produced by the injection of 1.5 ml. of turpentine into the pleural cavity of dogs before and after pancreatectomy. In other experiments, glucose was given intravenously, and in still others either glucose or insulin was injected into the pleural exudate. In all instances, blood and pleural exudate were examined for sugar, at first hourly and later daily. In no instance did inflammation cause any sudden or pronounced hyperglycemia. Glucose and nonprotein nitrogen constituents were higher in the diabetic than in the nondiabetic animals. Changes in their values in the blood and pleural exudate closely parallelled each other, and were not affected by the diabetes. The findings do not permit the conclusion originally brought forward by Menken that "gluconeogenesis occurs locally at the site of a chemically induced inflammation in the depancreatized dog."—*T.H. McG.*

RABINOWITCH, I. M., and MOUNTFORD, M.: Metabolism of sucrose: 11. *Am. J. Digest. Dis.* **14**: 315–322, 1947.

The authors measured blood sugar levels at one-minute intervals following the oral administration of various carbohydrates to patients with postinsulin hypoglycemia. Elevations in blood sugar were noted within one minute after sucrose; within two to three minutes after dextrose; and within three minutes after mixture of equal amounts of dextrose and levulose. Levulose did not raise the blood sugar five minutes after its administration. Sucrose ingestion resulted in a greater CO_2 expiration than that observed with dextrose. Following the ingestion of 50 grams of the sugars mentioned, normal and diabetic subjects demonstrated the greatest increase in blood sugar when dextrose was administered. In this study it was observed that serum inorganic phosphate decreased more rapidly, to lower levels, and remained low for a longer period, after ingestion of sucrose than with the other sugars. With 100 grams of sucrose the effect on the serum inorganic phosphate was even more marked. The authors feel that dextrose and levulose "do not alone account for the metabolism of sucrose" and therefore seek some unknown substance in the gastric contents to explain the phenomenon.—*H.S.G.*

WACHSTEIN, M.: Glycogen storage (von Gierke's) disease predominantly involving the heart; report of a case with histochemical phosphatase studies, *Am. J. M. Sc.* **214**: 401, 1947.

The author reports a case of von Gierke's disease in which large amounts of glycogen were demonstrated in the heart and the other organs by several histochemical methods, and in which, in addition, the distribution of alkaline and acid phosphatase in sections in various organs was investigated. This was the eighteenth proven case of von Gierke's disease with marked enlargement of the heart. Microscopic examination revealed glycogen storage in the cells of various organs, especially in those of the brain, the nerve cells in the myenteric plexus of the intestinal tract, and the Küpffer cells of the liver. The histochemical distribution of alkaline and acid phosphatase in the liver, the kidneys, the adrenals and the heart was normal in contrast to the findings of Thannhauser and coworkers (*J. Clin. Investigation* **19**: 681, 1940) who reported a marked decrease in alkaline phosphatase in the livers of three patients who died of the hepatic form of von Gierke's disease. There was marked hyperplasia of the Langerhans islands in the pancreas with preponderance of beta cells. The author speculates that this latter finding may be due to an increased demand for insulin.—*E.C.R., Jr.*

PITUITARY

BEASER, S. B.: Renal excretory function and diet in diabetes insipidus, *Am. J. M. Sc.* **213**: 441, 1947.

In diabetes insipidus in the dog, polyuria has been found to vary directly with the total daily osmillimolar excretion of sodium chloride and nitrogen (calculated as urea) of the urine. In man, likewise, these two constituents form the bulk of the various solids of the urine both in the normal person and in the patient with diabetes insipidus. The author made a study of the renal excretory function of human diabetes insipidus utilizing data from one patient studied by himself and from eight other patients studied by other investigators and reported in the literature. With these data, the author demonstrated quantitatively the low concentrating activity and reserve power of the kidney of patients with diabetes insipidus, and established a straight line relationship between the urine

volume and the total excreted quantities of salt and nitrogen expressed in osmillimols. This curve of renal excretion was found to be characteristic for each patient and was defined mathematically. The author proposes a new quantitative definition of severity in diabetes insipidus in terms of renal excretory dysfunction. The renal excretory curve of a diabetes insipidus patient provides a quantitative method of measuring the effect to be expected from dietary restriction or other procedures. It was demonstrated that maximal restriction of both salt and protein of the diet is necessary in order to attain maximal therapeutic benefit. The author believes that dietary control is worthy of a trial in every case, with or without posterior pituitary extract, according to the severity of the disease.—*E.C.R., Jr.*

THYROID

CRILE, G., JR.: Treatment of hyperthyroidism, *Canad. M.A.J.* **57**: 357, 1947.

Two hundred and eighteen cases of hyperthyroidism were treated with propylthiouracil, and 24 cases with methylthiouracil. In the latter group there were no toxic reactions. Two of the 218 patients treated with propylthiouracil had toxic reactions severe enough to cause discontinuation of treatment. The toxic reactions observed with the use of this drug included granulopenia (1 case), urticaria (2 cases), arthralgia (1 case), severe exfoliative dermatitis (1 case), nausea (1 case), and numbness of extremities (3 cases). Propylthiouracil was originally used in a daily dose of 75 mg., but this was inadequate. Dosage is now 200 mg. to 400 mg., and may have to be increased to 500 or 600 mg. Toxicity of the drug does not seem to increase with higher dosage. Methylthiouracil was used in doses up to 400 mg. daily. Remissions were obtained more promptly than with propylthiouracil. The question of permanency of remissions after withdrawal of the drug cannot be answered as yet. The need of intelligent cooperation of the patient, and of special experience with this type of therapy by the physician, is stressed. Toxic nodular goiters as well as very severe and large diffuse toxic goiters should be treated surgically. In the group of moderately severe diffuse toxic goiters the future role of surgical treatment will have to be evaluated in the light of extended experience.—*K.E.P.*

KEATING, F. R., JR.; POWER, M. H.; BERKSON J., and HAINES, S. F.: The urinary excretion of radioiodine in various thyroid states, *J. Clin. Investigation* **26**: 1138–1151, 1947.

The urinary excretion of radioiodine (I^{131}) was studied in 4 normal men; 10 euthyroid subjects who had papillary adenocarcinoma of the thyroid, grade I; 6 patients with myxedema, of whom 4 were untreated; and 16 patients with untreated, uncomplicated exophthalmic goiter. The usual tracer dose of I^{131} was 100 microcuries with a total of 100 micrograms of sodium iodide as carrier. Six-hour samples of urine were collected during the first 24 hours, and 24-hour samples thereafter for two more days. From the amount of I^{131} excreted in each of these specimens, excretion curves were constructed for each individual as well as for each group of subjects. By mathematical analysis it was possible to express quantitatively the differences in behavior of the ingested iodine between the euthyroid (this group includes the normal subjects and those with the thyroid tumors), hypothyroid and hyperthyroid subjects, and to arrive at four values of biological significance: 1) *The "renal fraction," or the portion of the ingested iodine primarily excreted in the urine.* In 72 hours, this amounted to approximately 65 per cent for the euthyroid

individuals, as contrasted with 85 per cent for the hypothyroid and 25 per cent for the exophthalmic subjects. 2) *The "disappearance rate," or the proportionate rate (per cent per hour) at which the radioiodine leaves the blood stream.* This was significantly decreased in myxedema and greatly increased in exophthalmic goiter. The accuracy of making such a calculation from the urinary excretion was checked in 4 cases by direct determination of the values for iodine in simultaneously collected blood samples. The close agreement between these two sets of determinations was highly significant. 3) *The "renal excretion rate," or the proportional rate at which the ingested iodine is excreted into the urine.* In per cent per hour, the mean renal excretion rate of all four groups was approximately the same, suggesting that differences in total excretion stand in close relationship to the rapidity of disappearance of iodine from the blood, and do not depend upon an altered status of renal function. The authors suggest that "one might expect to find reduced renal excretion of significant degree in hypothyroidism," and that a larger series of untreated patients should be studied to settle this point completely. 4) *The "collection rate," or the proportional rate of disappearance of radioiodine from the blood into tissues other than the kidney.* For rather obvious reasons, the collection rate is accepted by the authors as an index of the thyroid-collection rate. For the normal and euthyroid subjects this averaged 3.4 per cent per hour, decreased to one per cent per hour in myxedema, and increased some sixfold to an average of 23.5 per cent per hour in Graves' disease. In view of the results obtained, particularly the smoothness of the excretion curves, their exponential character, and the variations between the types of cases studied, the method of study used may afford "a means for determining the capacity of the kidneys to excrete iodine without regard to thyroid function, and of the thyroid to collect iodine without regard to variations in renal function." However, with the amounts of I^{131} used as a tracer in the present study, it is still necessary to recognize the possibility of biologic effects directly due to the concentration of radioactive material within the thyroid.—*T.H. McG.*

WINKLER, A. W.; MAN, E. B., and DANOWSKI, T. S.: Minimum dosage of thiourea, given together with iodine medication, necessary for the production and maintenance of a remission in hyperthyroidism, *J. Clin. Investigation* **26**: 446–452, 1947.

The production and maintenance of remissions of hyperthyroidism by the use of iodine, and of a combination of thiourea and iodine, have been studied in a group of 89 patients. The clinical condition of the patient and the level of serum-precipitable iodine (values greater than 8.0 micrograms per 100 cc.) have been utilized as the basis for the diagnosis of hyperthyroidism. Iodine was always administered in the form of Lugol's solution, five drops three times daily. In conjunction with this dose, it was found that 210 mg. and 280 mg. daily, respectively, of thiourea nearly always produced complete remission of the hyperthyroidism within from ten to twelve weeks of initiating treatment. Seventy mg. daily "proved to be somewhat less effective," as only 10 of 13 patients had responded by the end of twelve weeks. A daily intake of 15 mg. was insufficient to improve the status of the subjects observed. Symptoms of hypothyroidism were common at the two highest levels of dosage mentioned but were rare when 70 mg. were used daily, and did not occur at all when 15 mg. were employed. Iodine alone was ineffective in controlling the manifestations of hyperthyroidism, although 9 of 39 subjects showed a decline in the serum-precipitable iodine to normal levels while such treatment was being utilized. When iodine was employed in conjunction with thiourea, remissions could be maintained "for prolonged periods of time" with daily doses of thiourea ranging

from 25 mg. to 75 mg. When thiourea was stopped, relapse occurred with equal frequency in those who simultaneously discontinued therapy with iodine and those who did not. The authors conclude that "prolonged and perhaps indefinite, maintenance on thiourea and iodine is necessary to prevent recrudescence of hyperthyroidism." Several points in the discussion of their results justify emphasis. The very low concentration of thiourea attained in body fluids and tissues under the above treatment, and the relatively high concentration of iodine presumptively present in the thyroid, led the authors to conclude that the effect of thiourea is probably not one of mass action upon the available iodine. Moreover, since the iodine medication did "not interfere with the action of thiourea, even when the latter was present in trifling amounts," and since "their actions are additive," the conclusion may be drawn that "these substances act at quite different points." In the rest of the discussion, the authors assume that thiourea and thiouracil act in an identical fashion to "poison the enzyme system responsible" for the conversion of inorganic iodine to diiodotyrosine and thyroxine.—*T. H. McG.*